

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

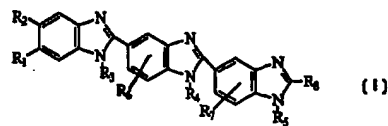
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C07D 235/18, 235/26, 235/28, 235/30, A61K 31/415, C07D 491/04, 235/02 //</b> <b>(C07D 491/04, 317:00, 235:00)</b>		<b>A1</b>	(11) International Publication Number: <b>WO 99/41241</b> (43) International Publication Date: <b>19 August 1999 (19.08.99)</b>
(21) International Application Number: <b>PCT/US99/02966</b> (22) International Filing Date: <b>12 February 1999 (12.02.99)</b> (30) Priority Data: <b>09/023,147</b> <b>12 February 1998 (12.02.98)</b> <b>US</b> (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application <b>US 09/023,147 (CIP)</b> <b>Filed on 12 February 1998 (12.02.98)</b> (71) Applicant (for all designated States except US): <b>RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY [US/US];</b> <b>Old Queens Building, Somerset and George Streets, New Brunswick, NJ 08903 (US).</b> (72) Inventors; and (75) Inventors/Applicants (for US only): <b>LAVOIE, Edmond, J. [US/US]; 3 Guilford Court, Princeton Junction, NJ 08550 (US). KIM, Jung, Sun [KR/KR]; Kyungnam-Hansin, Apartment 106-1606, Pugok-dong, Keumjung-gu, Pusan 609-232 (KR). RANGARAJAN, Meera [IN/US]; 14 Holly</b>		Court, Highland Park, NJ 08904 (US). LIU, Leroy, Fong [US/US]; 5 Fairacres Drive, Bridgewater, NJ 08807 (US). (74) Agent: <b>VIKSNINS, Ann, S.; Schwegman, Lundberg, Woessner &amp; Kluth, P.O. Box 2938, Minneapolis, MN 55402 (US).</b> (81) Designated States: <b>AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</b> <b>Published</b> <b>With international search report.</b>	

(54) Title: HETEROCYCLIC TOPOISOMERASE POISONS

## (57) Abstract

The invention provides a topoisomerase poison of formula (I) wherein R<sub>1</sub>-R<sub>8</sub> have any of the meanings defined in the specification, or a pharmaceutically acceptable salt thereof, as well as pharmaceutical compositions comprising a compound of formula (I) or a salt thereof, intermediates useful for preparing a compound of formula (I), and therapeutic methods comprising administering a compound of formula (I) or a salt thereof.



Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>
1	H	Phenyl	H	H	H	H	H	H
2	H	Phenyl	H	H	H	H	H	OH
3	H	Phenyl	H	H	H	H	H	SH
4	H	Phenyl	H	H	H	H	H	NH <sub>2</sub>
5	H	Phenyl	H	H	H	H	H	CF <sub>3</sub>
6	H	Phenyl	H	H	H	H	H	CH <sub>3</sub>
7	H	Phenyl	H	H	H	H	H	CH <sub>2</sub> OH
8	H	Phenyl	H	H	H	H	H	CH <sub>2</sub> NHCOCH <sub>3</sub>
9	H	Phenyl	H	H	H	H	H	CH <sub>2</sub> CH <sub>3</sub>
10	H	Phenyl	H	H	H	H	H	(CH <sub>2</sub> ) <sub>3</sub> OH
11	H	Phenyl	H	H	H	H	H	(CH <sub>2</sub> ) <sub>3</sub> NH <sub>2</sub>
12	H	Phenyl	H	H	H	H	H	(CH <sub>2</sub> ) <sub>3</sub> NHCOCH <sub>3</sub>
13	H	Phenyl	H	H	H	H	H	(CH <sub>2</sub> ) <sub>3</sub> OCH <sub>3</sub>
14	H	Phenyl	H	H	H	H	H	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>
15	Phenyl	OCH <sub>3</sub>	H	H	H	H	H	H
16	Phenyl	Phenyl	H	H	H	H	H	H
17	H	Br	H	H	H	H	H	H
18	H	Br	H	H	H	H	H	OH
19	H	Br	H	H	H	H	H	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>
20	Br	Br	H	H	H	H	H	H
21	OCH <sub>3</sub>	Br	H	H	H	H	H	H
22	Br	Br	H	H	H	H	H	CF <sub>3</sub>
23	p-Cl-phenyl	H	H	H	H	H	H	CF <sub>3</sub>

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## HETEROCYCLIC TOPOISOMERASE POISONS

5

### Government Funding

The invention described herein was made with government support under grant CA-39662 awarded by the National Cancer Institute. The United States Government has certain rights in the invention.

### Background of the Invention

10

DNA-topoisomerases are enzymes present in the nuclei of cells where they catalyze the breaking and rejoining of DNA strands, controlling the topological state of DNA. Recent studies also suggest that topoisomerases are involved in regulating template supercoiling during RNA transcription. There are two major classes of mammalian topoisomerases. DNA-topoisomerase-I catalyzes changes in the topological state of duplex DNA by performing transient single-strand breakage-union cycles. In contrast, mammalian topoisomerase II alters the topology of DNA by causing a transient enzyme bridged double-strand break, followed by strand passing and resealing. Mammalian topoisomerase II has been further classified as Type II  $\alpha$  and Type II  $\beta$ . The antitumor activity associated with agents which are topoisomerase poisons is associated with their ability to stabilize the enzyme-DNA cleavable complex. This drug-induced stabilization of the enzyme-DNA cleavable complex effectively converts the enzyme into a cellular poison.

15

Several antitumor agents in clinical use have potent activity as mammalian topoisomerase II poisons. These include adriamycin, actinomycin D, daunomycin, VP-16, and VM-26 (teniposide or epipodophyllotoxin).

20

In contrast to the number of clinical and experimental drugs which act as topoisomerase II poisons, there are currently only a limited number of agents which have been identified as topoisomerase I poisons. Camptothecin and its structurally-related analogs are among the most extensively studied topoisomerase I poisons. Recently, bi- and terbenzimidazoles (Chen et al., *Cancer Res.* 1993, 53, 1332-1335; Sun et al., *J. Med. Chem.* 1995, 38, 3638-3644; Kim et al., *J. Med. Chem.* 1996, 39, 992-998), certain

25

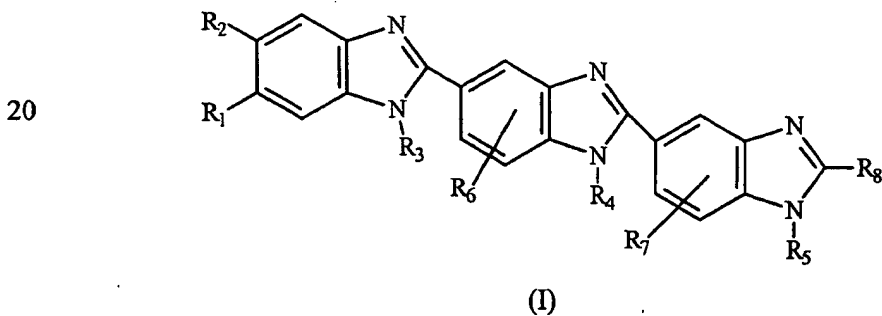
30

benzo[c]phenanthridine and protoberberine alkaloids and their synthetic analogs (Makhey et al., *Med. Chem. Res.* **1995**, *5*, 1-12; Janin et al., *J. Med. Chem.* **1975**, *18*, 708-713; Makhey et al., *Bioorg. & Med. Chem.* **1996**, *4*, 781-791), as well as the fungal metabolites, bulgarein (Fujii et al., *J. Biol. Chem.* **1993**, *268*, 13160-13165) and saintopin (Yamashita et al., *Biochemistry* **1991**, *30*, 5838-5845) and indolocarbazoles (Yamashita et al., *Biochemistry* **1992**, *31*, 12069-12075) have been identified as topoisomerase I poisons.

Presently, a need exists for novel and effective anti-cancer agents. In particular, there is also a need for anti-cancer agents that exhibit improved activity, fewer side-effects, or improved selectivity compared to existing agents.

#### Summary of the Invention

The present invention provides compounds that exhibit inhibitory activity against topoisomerase I, and compounds that are effective cytotoxic agents against cancer cells, including drug-resistant cancer cells. Accordingly there is provided a compound of the invention which is a compound of formula I:



25 wherein

$R_1$  and  $R_2$  are each independently hydrogen,  $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio,  $(C_1-C_6)$ alkanoyloxy, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, or heteroaryl $(C_1-C_6)$ alkyl; or  $R_1$  and  $R_2$  taken together are methylenedioxy; or  $R_1$  and  $R_2$  taken together are benzo;

$R_3$ ,  $R_4$ , and  $R_5$  are each independently selected from the group consisting of hydrogen,  $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_2-C_6)$ alkanoyloxy, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, and heteroaryl $(C_1-C_6)$ alkyl;

5  $R_6$  and  $R_7$  are each independently hydrogen,  $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, or  $(C_1-C_6)$ alkanoyloxy;

10  $R_8$  is hydroxy, halo, nitro, cyano, mercapto, carboxy,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy,  $-NR_aR_b$ , halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl, halo $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio,  $(C_1-C_6)$ alkanoyloxy, aryloxy, heteroaryloxy,  $SR_c$ , or  $S(O)_nR_d$ ; or  $R_8$  is  $(C_1-C_6)$ alkyl substituted by 1, 2, or 3 substituents independently selected  
15 from the group consisting of hydroxy, nitro, cyano, mercapto, carboxy,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy,  $-NR_aR_b$ , trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio,  $(C_1-C_6)$ alkanoyloxy, aryloxy, and heteroaryloxy;

$n$  is 1 or 2;

20 each of  $R_a$  and  $R_b$  is independently hydrogen,  $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, heteroaryl $(C_1-C_6)$ alkyl, arylcarbonyl, or heteroarylcarbonyl; or  $R_a$  and  $R_b$  together with the nitrogen to which they are attached are pyrrolidino, piperidino, or morpholino;

25  $R_c$  is  $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, heteroaryl $(C_1-C_6)$ alkyl, arylcarbonyl, halo $(C_1-C_6)$ alkyl, or heteroarylcarbonyl;

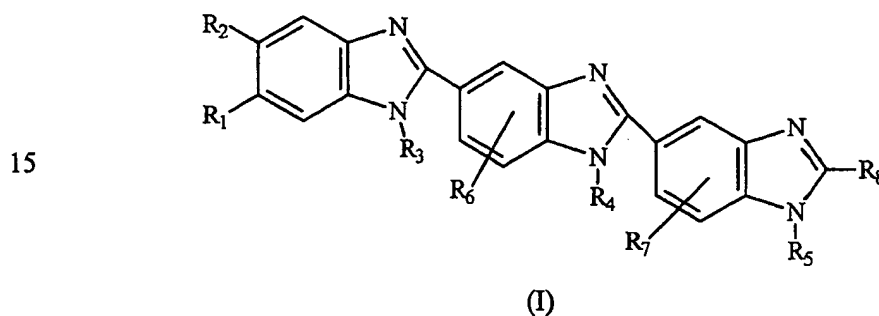
$R_d$  is  $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, heteroaryl $(C_1-C_6)$ alkyl, arylcarbonyl, halo $(C_1-C_6)$ alkyl,  $NR_eR_f$  or heteroarylcarbonyl; and  
30

$R_e$  and  $R_f$  are each independently hydrogen,  $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkanoyl, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, heteroaryl $(C_1-C_6)$ alkyl, arylcarbonyl, halo $(C_1-C_6)$ alkyl, or heteroarylcarbonyl;

wherein any aryl, heteroaryl, or benzo of  $R_1$ - $R_5$ ,  $R_6$ , and  $R_7$  may optionally be substituted by 1, 2, or 3 substituents independently selected from the group consisting of (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy(C<sub>1</sub>-C<sub>6</sub>)alkyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, and (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy;

or a pharmaceutically acceptable salt thereof.

The invention also provides a compound of the invention which is a compound of formula I:



wherein

20  $R_1$  and  $R_2$  are each independently (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy(C<sub>1</sub>-C<sub>6</sub>)alkyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy, aryl, heteroaryl, aryl(C<sub>1</sub>-C<sub>6</sub>)alkyl, or heteroaryl(C<sub>1</sub>-C<sub>6</sub>)alkyl; or  $R_1$  and  $R_2$  taken together are methylenedioxy;

$R_3$ ,  $R_4$ , and  $R_5$  are each independently selected from the group consisting of hydrogen, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>2</sub>-C<sub>6</sub>)alkanoyloxy, aryl, heteroaryl, aryl(C<sub>1</sub>-C<sub>6</sub>)alkyl, and heteroaryl(C<sub>1</sub>-C<sub>6</sub>)alkyl;

30  $R_6$  and  $R_7$  are each independently hydrogen, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy,

hydroxy(C<sub>1</sub>-C<sub>6</sub>)alkyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, or (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy; and

R<sub>8</sub> is hydrogen, (C<sub>1</sub>-C<sub>6</sub>)alkyl, aryl, or heteroaryl;

wherein any aryl or heteroaryl of R<sub>1</sub>-R<sub>5</sub> and R<sub>8</sub> may optionally be

5 substituted by 1, 2, or 3 substituents independently selected from the group consisting of (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy(C<sub>1</sub>-C<sub>6</sub>)alkyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, and (C<sub>1</sub>-  
10 C<sub>6</sub>)alkanoyloxy;

or a pharmaceutically acceptable salt thereof.

The invention also provides a pharmaceutical composition comprising a compound of formula I, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier.

15 The invention also provides a therapeutic method comprising inhibiting cancer cells by administering to a mammal (e.g. a human) in need of such therapy, an amount of a compound of formula I, or a pharmaceutically acceptable salt thereof, effective to inhibit said cancer cells.

The invention also provides a method comprising inhibiting  
20 cancer cells by contacting said cancer cells *in vitro* or *in vivo* with an amount of a compound of formula I, or a pharmaceutically acceptable salt thereof, effective to inhibit said cancer cells, i.e. to inhibit their activity, such as their ability to divide, migrate, or proliferate.

The invention also provides a compound of formula I for use in  
25 medical therapy (preferably for use in treating cancer, e.g. solid tumors; or for use as an antifungal agent), as well as the use of a compound of formula I for the manufacture of a medicament useful for the treatment of cancer, e.g. solid tumors, and the use of a compound of formula I for the manufacture of a medicament useful for the treatment of fungal infection.

30 The invention also provides processes and novel intermediates disclosed herein which are useful for preparing compounds of the invention. Some of the compounds of formula I are useful to prepare other compounds of formula I.

### Brief Description of the Figures

FIG. 1 shows the structure of representative compounds of the invention and other compounds (1, 6, 9, 14, 17, and 19).

FIG. 2 shows biological data for representative compounds of formula I.

5

### Detailed Description

The following definitions are used, unless otherwise described:

halo is fluoro, chloro, bromo, or iodo. Alkyl, alkoxy, etc. denote both straight and branched groups; but reference to an individual radical such as "propyl" embraces only the straight chain radical, a branched chain isomer such as "isopropyl" being specifically referred to. Aryl denotes a phenyl radical or an ortho-fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic. Heteroaryl encompasses a radical attached via a ring carbon of a monocyclic aromatic ring containing five or six ring atoms consisting of carbon and one to four heteroatoms each selected from the group consisting of non-peroxide oxygen, sulfur, and N(Y) wherein Y is absent or is H, O, (C<sub>1</sub>-C<sub>4</sub>)alkyl, phenyl or benzyl, as well as a radical of an ortho-fused bicyclic heterocycle of about eight to ten ring atoms derived therefrom, particularly a benzo-derivative or one derived by fusing a propylene, trimethylene, or tetramethylene diradical thereto.

20

It will be appreciated by those skilled in the art that compounds of the invention having a chiral center may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase) and how to determine topoisomerase poisoning activity or cytotoxic activity using the standard tests described herein, or using other similar tests which are well known in the art.

25

30

Specific and preferred values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other



defined values or other values within defined ranges for the radicals and substituents

Specifically, (C<sub>1</sub>-C<sub>6</sub>)alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, or hexyl; (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl can be cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl; (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkyl can be cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl, 2-cyclopropylethyl, 2-cyclobutylethyl, 2-cyclopentylethyl, or 2-cyclohexylethyl; (C<sub>1</sub>-C<sub>6</sub>)alkoxy can be methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, pentoxy, 3-pentoxy, or hexyloxy; (C<sub>1</sub>-C<sub>6</sub>)alkanoyl can be acetyl, propanoyl or butanoyl; halo(C<sub>1</sub>-C<sub>6</sub>)alkyl can be iodomethyl, bromomethyl, chloromethyl, fluoromethyl, trifluoromethyl, trichloromethyl, chlorodifluoromethyl, dichlorofluoromethyl, 2-chloroethyl, 2-fluoroethyl, 2,2,2-trifluoroethyl, or pentafluoroethyl; halo(C<sub>1</sub>-C<sub>6</sub>)alkanoyl can be trifluoroacetyl, chlorodifluoroacetyl, dichlorofluoroacetyl, 3,3,3-trifluoropropanoyl, or perfluoropropanoyl; hydroxy(C<sub>1</sub>-C<sub>6</sub>)alkyl can be hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropyl, 2-hydroxypropyl, 3-hydroxypropyl, 1-hydroxybutyl, 4-hydroxybutyl, 1-hydroxypentyl, 5-hydroxypentyl, 1-hydroxyhexyl, or 6-hydroxyhexyl; (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl can be methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, or hexyloxycarbonyl; (C<sub>1</sub>-C<sub>6</sub>)alkylthio can be methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, pentylthio, or hexylthio; (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy can be formyloxy, acetoxy, propanoyloxy, butanoyloxy, isobutanoyloxy, pentanoyloxy, or hexanoyloxy; aryl can be phenyl, indenyl, or naphthyl; and heteroaryl can be furyl, imidazolyl, triazolyl, triazinyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, pyrrolyl, pyrazinyl, tetrazolyl, pyridyl, (or its N-oxide), thienyl, pyrimidinyl (or its N-oxide), indolyl, isoquinolyl (or its N-oxide) or quinolyl (or its N-oxide).

A specific value for R<sub>1</sub> is hydrogen. Another specific value for R<sub>1</sub> is halo (e.g. bromo).

A specific value for R<sub>2</sub> is phenyl, optionally substituted by 1, 2, or 3 substituents independently selected from the group consisting of (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy,

halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, and (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy. Another specific value for R<sub>2</sub> is phenyl.

A specific value for each of R<sub>3</sub>, R<sub>4</sub>, and R<sub>5</sub> is hydrogen.

- 5 A specific value for R<sub>6</sub> or R<sub>7</sub> is (C<sub>1</sub>-C<sub>6</sub>)alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy(C<sub>1</sub>-C<sub>6</sub>)alkyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, or (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy. Another specific value for each of R<sub>6</sub> and R<sub>7</sub> is hydrogen.

- A specific value for R<sub>8</sub> is hydroxy, halo, nitro, cyano, mercapto, carboxy, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, -NR<sub>a</sub>R<sub>b</sub>, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, or (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy. Another specific value for R<sub>8</sub> is (C<sub>1</sub>-C<sub>6</sub>)alkyl substituted by 1, 2, or 3 substituents independently selected from the group consisting of hydroxy, nitro, cyano, mercapto, carboxy, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, -NR<sub>a</sub>R<sub>b</sub>, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, and (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy. Another specific value for R<sub>8</sub> is (C<sub>1</sub>-C<sub>6</sub>)alkyl substituted by hydroxy, nitro, cyano, mercapto, carboxy, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, -NR<sub>a</sub>R<sub>b</sub>, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, or (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy.

- A specific value for R<sub>8</sub> is hydroxy, halo, nitro, cyano, mercapto, carboxy, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkoxy, -NR<sub>a</sub>R<sub>b</sub>, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy, aryloxy, or heteroaryloxy; or R<sub>8</sub> is (C<sub>1</sub>-C<sub>6</sub>)alkyl substituted by 1, 2, or 3 substituents independently selected from the group consisting of hydroxy, nitro, cyano, mercapto, carboxy, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkoxy, -NR<sub>a</sub>R<sub>b</sub>, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy, aryloxy, and heteroaryloxy.

A specific value for R<sub>c</sub> is (C<sub>1</sub>-C<sub>6</sub>)alkyl.

A specific value for R<sub>d</sub> is (C<sub>1</sub>-C<sub>6</sub>)alkyl, or NR<sub>e</sub>R<sub>f</sub>.

- 30 A specific value for R<sub>e</sub> or R<sub>f</sub> is hydrogen, (C<sub>1</sub>-C<sub>6</sub>)alkyl, aryl, or aryl(C<sub>1</sub>-C<sub>6</sub>)alkyl.

A more specific value for R<sub>8</sub> is trifluoromethyl, chlorodifluoromethyl, dichlorofluoromethyl, 2,2,2-trifluoroethyl, perfluoroethyl,

trifluoromethoxy, cyano, halo, carboxy, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkanoyl, or S(O)<sub>n</sub>R<sub>d</sub>.

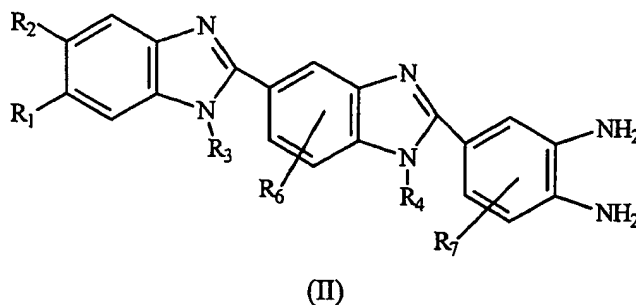
A preferred value for R<sub>1</sub> is hydrogen; for R<sub>2</sub> is phenyl; and for R<sub>8</sub> is halo or trifluoromethyl. A more preferred value for R<sub>8</sub> is trifluoromethyl.

5 A preferred group of compounds of formula I wherein R<sub>1</sub> and R<sub>2</sub> are each halo (e.g. bromo).

Processes for preparing compounds of formula I are illustrated by the following procedures in which the meanings of the generic radicals are as given above unless otherwise qualified.

10 A compound of formula I wherein R<sub>8</sub> is hydroxy can be prepared by treating an intermediate of formula II:

1

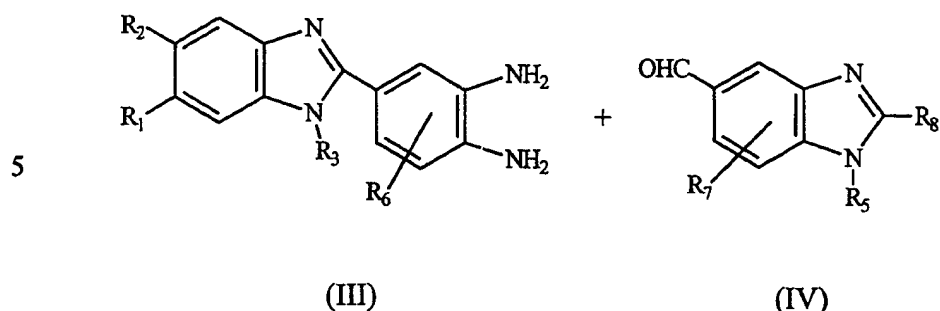


with urea, for example, using conditions similar to those described in Example 1.

A compound of formula I wherein R<sub>8</sub> is mercapto can be prepared  
 15 by treating an intermediate of formula II with ethylxanthic acid potassium salt, for example, using conditions similar to those described in Example 2.

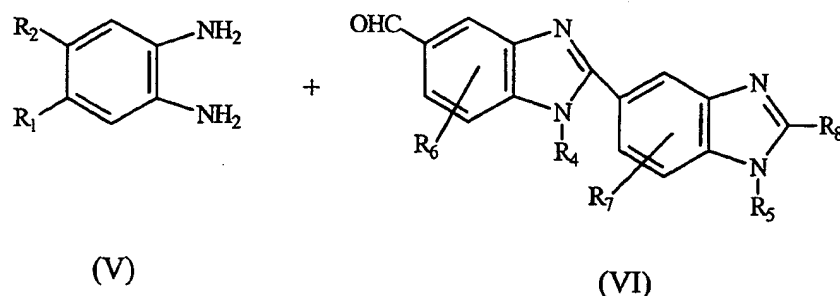
A compound of formula I wherein R<sub>8</sub> is amino can be prepared by treating an intermediate of formula II with cyanogen bromide, for example, using conditions similar to those described in Example 3.

20 Compounds of formula I can generically be prepared by reacting an intermediate diamine of formula III with an intermediate aldehyde of formula IV.



10 The reaction can conveniently be carried out under conditions similar to those described in the Examples (*e.g.*, Example 4).

Compounds of formula I can also generally be prepared by reacting an intermediate diamine of formula V with an aldehyde of formula VI.



The reaction can conveniently be performed under conditions similar to those  
15 described in the Examples (e.g. Example 12).

Compounds of formula I comprising a hydroxy group can be prepared from a corresponding compound of formula I comprising a methoxy group by treatment with boron tribromide, for example using conditions similar to those described in Example 7.

20                    Compounds of formula I comprising a amino group can be prepared from a corresponding compound of formula I comprising a acetamido group by hydrolysis using conditions similar to those described in Example 8.

Intermediates of formula II, III, IV, V, and VI wherein  $R_1$ - $R_8$  have any of the values, specific values, or preferred values defined herein for the corresponding radicals in a compound of formula I, are particularly useful for preparing compounds of formula I.

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compounds as salts may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate,  $\alpha$ -ketoglutarate, and  $\alpha$ -glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example, calcium) salts of carboxylic acids can also be made.

The compounds of formula I can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human patient in a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

Thus, the present compounds may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin;

excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance

of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thiomersal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

10 Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus 15 any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compounds may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination 20 with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, 25 optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

30 Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the compounds of formula I to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compounds of formula I can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the compound(s) of formula I in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

In general, however, a suitable dose will be in the range of from about 0.5 to about 100 mg/kg, e.g., from about 10 to about 75 mg/kg of body weight per day, such as 3 to about 50 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day.

The compound is conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

Ideally, the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.5 to about 75  $\mu$ M, preferably, about 1 to 50  $\mu$ M, most preferably, about 2 to about 30  $\mu$ M. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1-100 mg of the active ingredient. Desirable blood levels



may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the active ingredient(s).

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

The ability of a compound of the invention to effect topoisomerase I mediated DNA cleavage can be determined using pharmacological models that are well known to the art, for example, using a model like Test A described below.

**Test A. Topoisomerase I Cleavage Assay.**

Representative compounds of the invention were evaluated in a cleavage assay using recombinant topoisomerases I. This assay was preformed as described by B. Gatto et al. *Cancer Res.*, **1996**, *56*, 2795-2800. Human topoisomerase I was isolated as a recombinant fusion protein using a T7 expression system. Plasmid YEpG was purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation as described by Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning, a Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY 1982; pp 149-185. The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described by Liu, L. F.; Rowe, T. C.; Yang, L.; Tewey, K. M.; Chen, G. L. "Cleavage of DNA by mammalian topoisomerase II," *J. Biol. Chem.* **1983**, *258*, 15365. IC<sub>50</sub> values were calculated after 4 days of continuous drug exposure. Topoisomerase I cleavage values are reported as REC, Relative Effective Concentration (i.e., concentrations relative to compound 5, whose value is arbitrarily assumed as 1) that is able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I.

The cytotoxic effects of a compound of the invention can be determined using pharmacological models that are well known to the art, for example, using a model like Test B described below.

5    **Test B.**            Cytotoxicity Assay.

Cytotoxicity was determined using the MTT-microtiter plate tetrazolium cytotoxicity assay (MTA) (See Chen A.Y. et al. *Cancer Res.* **1993**, 53, 1332; Mosmann, T. J., J. *Immunol. Methods* **1983**, 65, 55; and Carmichael, J. et al. *Cancer Res.* **1987**, 47, 936). The human lymphoblast RPMI 8402 and its  
10    camptothecin-resistant variant cell line, CPT-K5 were provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan) (see Andoh, T.; Okada, K. "Drug resistance mechanisms of topoisomerase I drugs," *Adv. in Pharmacology* **1994**, 29B, 93. The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% CO<sub>2</sub> and  
15    maintained by regular passage in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). For determination of IC<sub>50</sub>, cells were exposed continuously with varying concentrations of drug and MTT assays were performed at the end of the fourth day.

20                    Data from Test A and Test B is shown in Figure 2 for representative compounds of the invention.

Using a protocol similar to Test B, the cytotoxicity of compound 5 and compound 22 was evaluated in other tumor cell lines. The data is presented in Table 1.

Table 1

	Compound	Cell Line	Organ Site	Cytotoxicity IC50 ( $\mu$ M)
5	5	MDA-MB-435	Mammary	0.08
	5	MCF7	Mammary	>30
	5	OVCAR-3	Ovarian	0.26
	5	SK-OV-3	Ovarian	0.55
	5	PC-3	Prostate	0.06
10	5	DU-145	Prostate	0.59
	5	SF-268	Glioblastoma	0.26
	5	SF-295	Giloblastoma	0.23
	5	NCI-H82	Large Cell Lung	0.26
	5	NCI-H23	Small Cell Lung	2.55
15	5	HT29	Colon	2.65
	5	KB3-1	Epidermal Oral	0.01
	5	KBV-1	Oral MDR1	11.0
	22	MDA-MB-435	Mammary	0.15
	22	MCF7	Mammary	>100
20	22	OVCAR-3	Ovarian	6.31
	22	SK-OV-3	Ovarian	1.36
	22	PC-3	Prostate	0.23
	22	DU-145	Prostate	2.04
	22	SF-268	Glioblastoma	2.27
25	22	SF-295	Giloblastoma	0.25
	22	NCI-H82	Large Cell Lung	0.79
	22	NCI-H23	Small Cell Lung	>100
	22	HT29	Colon	>100

Compounds of formula I are potent topoisomerase I poisons.

Additionally, compounds of formula I have been shown to exhibit cytotoxic activity against RPMI 8402 cancer cells, camptothecin resistant CPT-K5 cells, and numerous other tumor cells. Accordingly, compounds of formula I are

useful as cytotoxic agents, for the treatment of cancers, and in particular, solid mammalian tumors or hematologic malignancies. Compounds of the invention are also useful as pharmacological tools for *in vitro* and *in vivo* study of topoisomerase function and activity.

5                   As used herein, the term "solid mammalian tumors" includes cancers of the head and neck, lung, mesothelioma, mediastinum, esophagus, stomach, pancreas, hepatobiliary system, small intestine, colon, rectum, anus, kidney, ureter, bladder, prostate, urethra, penis, testis, gynecological organs, ovarian, breast, endocrine system, skin central nervous system; sarcomas of the  
10 soft tissue and bone; and melanoma of cutaneous and intraocular origin. The term "hematological malignancies" includes childhood leukemia and lymphomas, Hodgkin's disease, lymphomas of lymphocytic and cutaneous origin, acute and chronic leukemia, plasma cell neoplasm and cancers associated with AIDS. The preferred mammalian species for treatment are humans and  
15 domesticated animals.

                  The compounds are also expected to exhibit some of the other bioactivities observed for topoisomerase inhibitors, such as antibacterial, antifungal, antiprotozoal, antelmintic and/or antiviral activity. In particular, the compounds may exhibit antifungal activity. Thus, the invention also provides a  
20 therapeutic method to treat fungal infection comprising administering to a mammal afflicted with a fungal infection, an effective antifungal amount of a compound of the formula I, or a pharmaceutically acceptable salt thereof.

                  The invention will now be illustrated by the following non-limiting Examples, wherein unless otherwise stated: melting points were  
25 determined with a Thomas-Hoover Unimelt capillary melting point apparatus; column chromatography refers to flash chromatography conducted on SiliTech 32-63  $\mu\text{m}$ , (ICN Biomedicals, Eschwege, Ger.) using the solvent systems indicated; infrared spectral data (IR) were obtained on a Perkin-Elmer 1600 Fourier transform spectrophotometer and are reported in  $\text{cm}^{-1}$ ; proton ( $^1\text{H}$  NMR)  
30 and carbon ( $^{13}\text{C}$  NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer; NMR spectra (200 MHz  $^1\text{H}$  and 50 MHz  $^{13}\text{C}$ ) were recorded in the deuterated solvent indicated with chemical shifts reported in  $\delta$  units downfield from tetramethylsilane (TMS); coupling constants

are reported in hertz (Hz); mass spectra were obtained from Washington University Resource for Biomedical and Bio-organic Mass Spectrometry within the Department of Chemistry at Washington University, St. Louis, MO; and combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and were within  $\pm 0.4\%$  of the theoretical value.

### EXAMPLES

**Example 1. 5-Phenyl-2-[2'-(2"-hydroxybenzimidazol-5"-yl)benzimidazol-5'-yl]benzimidazole (2).**

10                   5-Phenyl-2-[2'-(3,4-diaminophenyl)benzimidazol-5'-yl]benzimidazole, (29.7 mg, 0.07 mmol) and urea (6 mg, 0.1 mmol) were dissolved in DMF (0.4 ml). The mixture was refluxed at 150 °C for 7 hours. The cooled reaction mixture when concentrated *in vacuo* and washed with water provided 27.6 mg (88%) of pure yellow solid; mp > 280 °C; IR (KBr) 3382, 15  
3133, 1693, 1475, 1444, 1279; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH)  $\delta$  7.3 (d, 1H, J= 8.14), 7.46-7.80 (m, 3H), 7.80 (d, 2H, J=8.1), 7.89-8.01 (m, 4H), 8.05-8.14 (m, 2H), 8.32 (d, 1H, J=8.34), 8.63 (s, 1H), 11.35 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH)  $\delta$  108.07, 109.55, 111.78, 114.03, 114.57, 114.62, 115.3, 120.21, 122.53, 125.45, 125.82, 127.42, 128.1, 129.26, 130.81, 131.59, 132.66, 132.87, 135.15, 135.33, 139.20, 139.61, 149.32, 153.14, 155.71; HRMS (FAB)  
20 calculated for C<sub>27</sub>H<sub>19</sub>N<sub>6</sub> (MH<sup>+</sup>) 443.1620 found 443.1625.

The intermediate 5-phenyl-2-[2'-(3,4-diaminophenyl)-benzimidazol-5'-yl]benzimidazole was prepared as follows.

25   a.   **5-Phenyl-2-[2'-(3,4-diaminophenyl)benzimidazol-5'-yl]benzimidazole.**

A solution of 5-phenyl-2-[2'-(3,4-dinitrophenyl)benzimidazol-5'-yl]benzimidazole (75 mg, 0.16 mmol) in ethyl acetate (50 mL) was reduced by hydrogenation over 10% Pd/C (15 mg) for 90 minutes. The resulting solution was passed through a bed of Celite and the ethyl acetate was removed to give the  
30 diamine, which was used without further purification.

The starting 5-phenyl-2-[2'-(3,4-dinitrophenyl)benzimidazol-5'-yl]benzimidazole can be prepared as described by J.S. Kim et al. J. Med. Chem. 1997, 40, 2818-2824.

**Example 2. 5-Phenyl-2-[2'-(2''-mercaptobenzimidazol-5''-yl)benzimidazol-5'-yl] benzimidazole (3).**

5-Phenyl-2-[2'-(3,4-diaminophenyl)benzimidazol-5'-yl]-benzimidazole, (59.4 mg, 0.14 mmol) and ethylxanthic acid potassium salt (25.1 mg, 0.16 mmol) were refluxed in ethanol (1 ml) and distilled water (0.1 ml) overnight. The cooled reaction mixture was acidified to pH 3 with glacial acetic acid, volume condensed *in vacuo* and purified directly by column chromatography. Elution with (40-100%) ethyl acetate/n-hexanes provided 69% of yellow solid; mp > 280 °C; IR (KBr) 3089, 2926, 2851, 1712, 1624, 1549, 1449, 1380, 1274, 1186, 1079; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 7.33-7.42 (m, 2H), 7.48-7.63 (t, 4H), 7.71-7.82 (m, 4H), 7.89 (s, 1H), 8.04-8.06 (m, 1H), 8.1-8.24 (m, 1H), 8.42 (d, 1H, J=2.4); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 107.37, 107.15, 109.2, 109.68, 111.81, 111.73, 113.31, 113.53, 114.57, 114.64, 114.68, 115.56, 125.26, 127.44, 128.16, 129.33, 131.64, 132.93, 133.27, 139.1, 136.61, 139.62, 149.51, 152.94, 171.13; HRMS (FAB) calculated for C<sub>27</sub>H<sub>19</sub>N<sub>6</sub>S (MH<sup>+</sup>) 459.1392, found 459.1403.

**Example 3. 5-Phenyl-2-[2'-(2''-aminobenzimidazol-5''-yl)benzimidazol-5'-yl] benzimidazole (4).**

5-Phenyl-2-[2'-(3,4-diaminophenyl)benzimidazol-5'-yl]benzimidazole, (66 mg, 0.16 mmol) was dissolved in DMF (0.2 ml) and methanol (1 ml), and was added to cyanogen bromide (10% solution in water, 0.33 ml, 0.63 mmol). The reaction mixture was stirred overnight at room temperature. The mixture was concentrated under reduced pressure. Compound was recrystallized from methanol to give 47% of pale brown solid; mp > 280 °C; IR (KBr) 3352, 3052, 2926, 1680, 1624, 1574, 1461, 1261, 679; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 7.46-7.62 (m, 3H), 7.79 (d, 2H, J=7.26), 7.87-7.96 (m, 2H), 7.99-8.01 (m, 2H), 8.20-8.26 (m, 2H), 8.31 (s, 1H), 8.72 (d, 2H, J=9.12); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 107.38, 110.89, 111.75, 111.78, 114.67, 115.13, 115.24, 115.92, 123.59, 125.58, 126.84, 127.5, 127.53, 127.57, 127.76, 128.2, 129.43, 130.86, 131.87, 133.13, 138.74, 139.74, 150.10, 152.14, 153.42; HRMS (FAB) calculated for C<sub>27</sub>H<sub>20</sub>N<sub>7</sub> (MH<sup>+</sup>) 442.1780 found 442.1776.

**Example 4. 5-Phenyl-2-[2'(2"-trifluoromethylbenzimidazol-5"-yl)benzimidazol-5'-yl]benzimidazole (5).**

5-Phenyl-2-[3,4-diaminophenyl]benzimidazole (0.160 g, 0.53 mmol) was heated with 5-formyl-2-trifluoromethylbenzimidazole (0.12 g, 0.54 mmol) in nitrobenzene (4 mL) at 145 °C overnight. Nitrobenzene was removed using a Kugelrohr and the compound purified by column chromatography. (1-16%) methanol/ethyl acetate provided 40% pure yellow compound; mp > 280 °C; IR (KBr) 3047, 2927, 1698, 1626, 1543, 1440, 1287, 1158; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>+ 3 drops CF<sub>3</sub>COOH) δ 7.44-7.59 (m,3H), 7.79 (d,2H,J=7.04), 7.87-8.01 (m,2H), 8.05-8.09 (m,2H), 8.15-8.19 (m,1H), 8.29-8.35 (m,2H), 8.70 (m,2H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>+ 3 drops CF<sub>3</sub>COOH) δ 111.79, 114.68, 114.92, 115.85, 117.53, 117.58, 118.37, 118.41, 119.58, 120.46, 124.14, 124.79, 125.71, 125.74, 127.47, 128.18, 129.36, 131.73, 132.99, 134.93, 137.44, 138.97, 139.02, 139.07, 139.66, 140.34, 149.81, 153.46; HRMS (FAB) calculated for C<sub>28</sub>H<sub>18</sub>N<sub>6</sub>F<sub>3</sub> (MH<sup>+</sup>) 495.1545 found 495.1543.

The intermediate 5-formyl-2-trifluoromethylbenzimidazole was prepared as follows.

a. **3,4-Diaminobenzonitrile.** 4-Amino-3-nitrobenzonitrile (3 g, 18.4 mmol) in ethyl acetate (120 ml) was reduced by hydrogenation using 45 psi of H<sub>2</sub> and 10 % Pd-C (300 mg) for 1.5 hr. After passing through a bed of celite, the solvent was removed *in vacuo* and 2.43 g (99 %) of white solid was obtained; mp 144-145 °C; IR (KBr) 3431, 2726, 2211, 1861, 1631, 1311, 1149, 722; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.18-3.87 (brs, 4H), 6.68 (d, 1H, J=8.06), 6.94 (d,1H,J=1.74), 7.02-7.27 (dd,1H,J=1.78, 8.06); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 100.27, 115.86, 119.52, 122.19, 125.66, 135.94, 142.52; The crude diamine obtained was typically used without further purification.

b. **5-Cyano-2-trifluoromethylbenzimidazole.** 3,4-Diaminobenzonitrile (200 mg, 1.5 mmol) was refluxed with trifluoroacetic acid (0.3 ml) for 6 hours. The mixture was neutralized with 2-N NaOH and extracted with ethyl acetate. The ethyl acetate layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Column with (0-10 %) ethyl acetate/n-hexanes gave 88% of pure white

compound; mp 182-183 °C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 7.72 - 7.77 (1H, dd, J=1.46, 8.56), 7.88 (d, 1H, 8.42), 8.35 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 106.47, 106.64, 112.36, 117.31, 118.09, 119.44, 123.53, 123.81, 127.47; Anal. calculated for C<sub>9</sub>H<sub>4</sub>N<sub>3</sub>F<sub>3</sub>: C; 51.20, H; 1.91, N; 19.90, 5 found C; 51.45, H; 2.03, N; 19.68.

c. **5-Formyl-2-trifluoromethylbenzimidazole.** 5-Cyano-2-trifluoromethyl-benzimidazole (196 mg, 0.93 mmol) was refluxed with HCOOH (14 ml), H<sub>2</sub>O (5 ml) and Ni-Al (0.9 g) catalyst for 6 hours. The hot reaction mixture was filtered through celite and concentrated *in vacuo*. The solution was basified with 2N NaOH. Extraction with ethyl acetate followed by drying over anhydrous sodium sulfate and concentration of the ethyl acetate layer *in vacuo* gave the crude aldehyde. Purification was achieved by column chromatography. Elution with 10/90 ethyl acetate/n-hexanes gave 41% of pure white compound; 10 mp 178-179 °C; IR (KBr) 3210, 2737, 1699, 1552, 1328, 1187 (d), 986; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 7.87-7.95 (m, 2H), 8.34 (s, 1H), 10.11 (s, 1H). <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 106.63, 112.35, 116.72, 118.08, 121.87, 123.80, 124.04, 132.95, 192.65; HRMS (EI) calculated for C<sub>9</sub>H<sub>5</sub>N<sub>2</sub>OF<sub>3</sub> *m/z* 214.0354 found 214.0348.

20 The intermediate 4-amino-3-nitrobenzonitrile used in subpart a above is commercially available (Aldrich Chemical Company)

The intermediate 5-phenyl-2-[3,4-diaminophenyl]benzimidazole was prepared as follows.

25

d. **5-Phenyl-2-[3,4-diaminophenyl]benzimidazole.** 5-Phenyl-2-[3,4-nitrophenyl]benzimidazole (580 mg, 1.6 mmol) was dissolved in 100 ml ethyl acetate and hydrogenation was carried out using 10% Pd/C (100 mg) for 1.5 hours. The reaction mixture was passed through a bed of celite and the bed 30 washed with methanol. The washings were concentrated *in vacuo* to yield (477 mg, 1.59 mmol) of the crude diamine in 98 % yield. The diamine was not characterized but used without purification; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops



CF<sub>3</sub>COOH)  $\delta$  4.70 (brs, 2H), 4.96 (brs, 2H), 6.62 (d, 1H, 8.02), 7.37 (m, 8H), 7.53 (m, 2H).

**Example 5. 5-Phenyl-2-[2'-(2"-hydroxymethylbenzimidazol-5"-yl)benzimidazol-5'-yl]benzimidazole (7).**

5-Phenyl-2-[3,4-diaminophenyl]benzimidazole (178 mg, 0.6 mmol) and 5-formyl-2-hydroxymethyl benzimidazole (104.2 mg, 0.6 mmol) were condensed in nitrobenzene (10 ml) overnight at 145 °C. Nitrobenzene was removed from the reaction mixture with a Kugelrohr and the compound was purified directly by column chromatography. Elution with (1-18 %) methanol/ethyl acetate provided 62% of yellow solid; mp > 280 °C; IR (Nujol) 3406, 2922, 2725, 1631, 1553, 1461, 1377; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH)  $\delta$  5.1 (s, 2H), 7.45-7.59 (m, 3H), 7.79 (d, 2H, J=6.96), 8.23-8.28 (m, 1H), 8.47 (d, 1H, J=8.59), 8.68 (s, 2H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH)  $\delta$  55.80, 111.69, 113.68, 114.59, 115.32, 115.77, 116.21, 118.31, 123.77, 124.52, 125.21, 125.61, 127.47, 127.49, 127.54, 128.14, 129.38, 131.64, 131.89, 132.91, 133.54, 137.66, 138.86, 139.68, 140.13, 150.25, 153.37, 157.62; HRMS (FAB) calculated for C<sub>28</sub>H<sub>21</sub>N<sub>6</sub>O (MH<sup>+</sup>) 457.1777 found 457.1774.

The intermediate 5-formyl-2-hydroxymethyl benzimidazole was prepared as follows.

**a. 5-Cyano-2-hydroxymethylbenzimidazole. 3,4**

Diaminobenzonitrile (0.2 g, 1.50 mmol) was heated with glycolic acid (0.18 g, 2.37 mmol) in HCl (1.5 ml of 4N) for 2 hours. After neutralization with 2M sodium carbonate the product was extracted into ethyl acetate, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to give a white colored solid. The compound was purified by column chromatography. Elution with (75-100%) ethyl acetate/n-hexanes gave 65.5% (133 mg, 0.76 mmol) of pure compound; mp 172-173 °C; IR (Nujol) 3350, 2928, 2219, 1621, 1536, 1302, 1217, 1036, 815; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH)  $\delta$  4.88 (s, 2H), 7.52-7.57 (dd, 1H, J=1.46, 8.38), 7.69 (d, 1H, J=8.36), 7.95 (s, 1H), <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH)  $\delta$  55.81, 107.99, 115.68, 118.64, 119.50, 129.00, 131.50, 134.39; HRMS (EI) calculated for C<sub>9</sub>H<sub>7</sub>N<sub>3</sub>O (m/z) 173.0590 found 173.0590.

b. **5-Formyl-2-hydroxymethylbenzimidazole.** Ni-Al (1.2 g) was added to a solution of 5-cyano-2-hydroxymethylbenzimidazole (0.17 g, 0.97 mmol) in HCOOH (17 ml) and water (1.6 ml). The reaction mixture was heated at 95 °C for 5 hours. The hot mixture was filtered through a bed of celite and the reaction flask and the celite bed rinsed with water and then methanol. The combined solution was concentrated *in vacuo*. After addition of water to this residue, a white precipitate was formed. The pH of this suspension was adjusted to 9 by dropwise addition of 2N NaOH. The product was obtained by extraction with ethyl acetate. The ethyl acetate extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to give a pure white product (61% yield); mp 189-190 °C; IR (KBr) 3309, 2924, 1674, 1619, 1434, 1291, 1073, 808; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 5.07 (s, 2H), 7.94 (d, 1H, J=8.68), 8.04-8.09 (dd, 1H, J=1.34, 8.51), 8.32 (s, 1H), 10.4 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 55.76, 114.95, 116.81, 126.03, 131.45, 133.89, 135.11, 192.31; HRMS (EI) calculated for C<sub>9</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub> m/z 176.0586 found 176.0586..

**Example 6. 5-Phenyl-2-[2'-[2''-[2-(N-benzoyl)aminomethyl]benzimidazol-5''-yl]benzimidazol-5'-yl]benzimidazole (8).**

5-Phenyl-2-[3,4-diaminophenyl]benzimidazole (75 mg, 0.25 mmol) and 5-formyl-2-[(N-benzoyl)aminomethyl]benzimidazole (70 mg, 0.25 mmol) were heated in nitrobenzene (6 ml) at 145 °C overnight. Nitrobenzene was removed with a Kugelrohr and the compound was loaded on a column. (2-20%) methanol/ethyl acetate gave 45% yellowish compound; mp > 280 °C; IR (KBr) 3204, 1637, 1542, 1442, 1384, 1292, 1026, 818; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 7.46-7.66 (m, 6H), 7.80 (d, 2H, J=7.06), 7.87-8.1 (m, 7H), 8.21-8.25 (m, 1H), 8.43-8.48 (m, 1H), 8.67 (s, 2H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 36.65, 107.37, 111.73, 114.53, 114.58, 115.26, 115.49, 116.01, 119.47, 122.49, 124.81, 125.56, 126.72, 127.41, 127.83, 128.04, 128.07, 128.54, 129.25, 131.56, 131.87, 132.12, 132.84, 133.17, 134.11, 136.21, 137.76, 139.08, 139.66, 149.67, 152.62, 155.64, 167.60; HRMS (FAB) calculated for C<sub>35</sub>H<sub>26</sub>N<sub>7</sub>O (MH<sup>+</sup>) 560.2199 found 560.2209.

The intermediate 5-formyl-2-[(N-benzoyl)aminomethyl]-benzimidazole was prepared as follows.

- a. **5-Cyano-2-[(N-benzoyl)aminomethyl]benzimidazole.** 3,4-Diaminobenzonitrile (250 mg, 1.88 mmol) was finely ground in a mortar with (0.34 g, 1.9 mmol) hippuric acid and intimately mixed and then carefully fused for 3 hours. The temperature was then raised to 160 °C when water was evolved as bubbles. After cooling the glassy mass was dissolved in ethyl acetate and purified by column chromatography. (50-100%) ethyl acetate/n-hexanes yielded 86 % of pale buff colored compound; mp 131-132 °C; IR (Nujol) 2826, 2221, 1635, 1313, 1019, 814; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 4.97 (d, 2H, J=5.22), 7.52-7.61 (m, 3H), 7.91-7.99 (m, 4H), 8.37 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 36.80, 107.73, 115.72, 118.81, 119.67, 127.83, 128.61, 128.64, 128.67, 128.78, 132.15, 132.20, 13322, 135.01, 156.13, 167.51; HRMS (EI) calculated for C<sub>16</sub>H<sub>12</sub>N<sub>4</sub>O *m/z* 276.1011 found 276.1012.
- b. **5-Formyl- 2-[(N-benzoyl)aminomethyl]benzimidazole.** 5-Cyano-2-[(N-benzoyl)aminomethyl]benzimidazole (0.33 g, 1.2 mmol) was dissolved in formic acid (18 ml). Water (5 ml) and Ni-Al (1.14 g) were added to it. The mixture was heated under nitrogen at 95° C for 6 hours and while hot passed through a celite bed. The bed was washed with methanol and the washings concentrated *in vacuo*. The pH of the solution was adjusted with 2N NaOH to 9.0 and extracted with ethyl acetate. The extractions were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The residue was purified over column chromatography using (50-100 %) ethyl acetate/n-hexanes to give 26 % of pure cream colored product; mp 108-109° C; IR (KBr) 3314, 2933, 1687, 1646, 1539, 1289, 806; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 4.99 (d, 1H, J=5.12), 7.54-7.63 (m, 4H), 7.93-8.07 (m, 3H), 8.34 (s, 1H), 10.15 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 36.83, 115.10, 117.18, 125.80, 127.85, 128.71, 132.26, 132.33, 133.2, 133.68, 155.77, 167.54, 192.54; HRMS (EI) calculated for C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub> *m/z* 279.1008 found 279.1003.

30

**Example 7. 5-Phenyl-2-[2'-(2''-(2-hydroxyethyl)benzimidazol-5''-yl)]benzimidazol-5'-yl]benzimidazole (10).**

5-Phenyl-2-[2'-[2''-(2-methoxyethyl)benzimidazol-5''-yl]benzimidazol-5'-yl]benzimidazole (30 mg, 0.06 mmol, Example 10) was suspended in freshly distilled ethyl acetate (30 ml) to which was added 10 equivalence of BBr<sub>3</sub> (0.62 ml, 1.0M) at -78 °C. After stirring at room temperature overnight, the reaction was quenched by adding water (10 ml). The mixture was basified to pH 9.0 with ammonium hydroxide and extracted with ethyl acetate and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crude mixture was separated on a chromatotron, (0-30%) methanol/ethyl acetate to give 10.6 mg of the product in 33 % yield as a brown solid. mp > 280 °C; IR (KBr) 3401, 3150, 2876, 1632, 1540, 1442, 1388, 1268, 1013, 823; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>+ 3 drops CF<sub>3</sub>COOH) δ 3.35 (t,2H), 3.98 (t,2H), 7.42-7.56 (m,3H), 7.76 (d,2H,J=7.7), 7.85-7.98 (m,2H), 8.06-8.19 (m,3H), 8.33 (d,1H, J=8.5), 8.44 (d,1H, J=8.8), 8.73 (s,2H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>+ 3 drops CF<sub>3</sub>COOH) δ 30.75, 58.09, 111.71, 114.36, 114.53, 115.28, 115.98, 116.02, 118.28,119.43, 122.37, 124.71, 124.76, 125.43, 125.73, 127.40, 128.1, 129.25, 131.55, 131.64, 132.83, 133.95, 135.26, 137.79, 139.06, 139.64, 149.67, 152.63, 156.21; HRMS (FAB) calculated for C<sub>29</sub>H<sub>23</sub>N<sub>6</sub>O (MH<sup>+</sup>) 471.1933 found 471.1935

**Example 8. 5-Phenyl-2-[2'-[2''-(2-aminoethyl)benzimidazol-5''-yl]benzimidazol-5'-yl]benzimidazole (11).**

5-Phenyl-2-[2'-[2''-(2-acetamidoethyl)benzimidazol-5''-yl]benzimidazol-5'-yl]benzimidazole (35 mg, 0.07 mmol, Example 9) was hydrolyzed with 2N HCl (5 ml) at 110°C for 3 hours. The cooled reaction mixture was basified with ammonium hydroxide to pH 9.0 and purified by column chromatography. Elution with 50:50 methanol/ethyl acetate provided 75% yellow solid; mp > 280 °C; IR (Nujol) 3375, 2719, 1553, 1461,1377; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>+ 3 drops CF<sub>3</sub>COOH) δ 3.45-3.52 (m,4H), 7.45-7.56 (m,2H), 7.79(d, 2H, J=7.04), 7.88-8.00 (m,2H), 8.07-8.27 (m,5H),8.42 (d,1H, J= 8.92), 8.68 (d,2H,J=4.76); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>+ 3 drops CF<sub>3</sub>COOH) δ 25.52, 36.36,111.74, 113.77, 114.62, 115.33, 115.76, 116.17, 118.33, 123.75, 123.94, 124.75, 125.61, 127.48,128.16, 129.39; 131.71, 132.98, 133.11, 134.77, 137.73, 138.84, 139.71, 140.12, 150.34, 153.43, 153.61; HRMS (FAB) calculated for C<sub>27</sub>H<sub>19</sub>N<sub>6</sub>O (MH<sup>+</sup>) 470.2093 found 470.2089.

**Example 9. 5-Phenyl-2-[2'-(2''-(2-acetamidoethyl)benzimidazol-5''-yl)]benzimidazol-5'-yl] benzimidazole (12).**

5-Phenyl-2-[3,4-diaminophenyl]benzimidazole (124 mg, 0.41 mmol) and 5-formyl-2-(2-acetamidoethyl)benzimidazole (95 mg, 0.41 mmol) were condensed in nitrobenzene (7 ml) overnight at 145 °C. Nitrobenzene was removed with the aid of a Kugelrohr and the compound purified directly by flash column chromatography. Elution with (1-15 %) methanol/ethyl acetate provided 69% of yellow solid; mp > 280 °C; IR (KBr) 3064, 2954, 2862, 1654, 1556, 1442, 1286; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 1.81 (s,3H), 3.18-3.35 (m,2H), 3.61-3.64 (m,2H), 7.45-7.6 (m,3H), 7.79 (d,2H,J=7.04 ), 7.87-8.00 (m,2H), 8.07-8.30 (m,4H), 8.46 (d,1H,J=8.5), 8.71 (s,2H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 22.59, 27.89, 36.41, 111.73, 113.66, 114.59, 115.22, 115.69, 116.17, 118.61, 124.04, 125.22, 125.66, 127.47, 127.56, 128.14, 128.18, 129.36, 131.62, 131.84, 132.89, 133.69, 137.10, 138.94, 139.54, 139.68, 150.16, 153.24, 155.40, 158.47, 170.27; HRMS (FAB) calculated for C<sub>31</sub>H<sub>25</sub>N<sub>7</sub>O (MH<sup>+</sup>) 512.2199 found 512.2201.

The intermediate 5-formyl-2-(2-acetamidoethyl)benzimidazole was prepared as follows.

a. **5-Cyano-2-(2-aminoethyl)benzimidazole.** A solution of 3,4-diaminobenzonitrile (1g, 7.52 mmol) and β-alanine (1.0 g, 11.3 mmol) in HCl (8 ml, 6N) was refluxed for 24 hrs. At this time the diamine was barely detectable by TLC. After neutralization with 2N NaOH, the compound was directly loaded on the column. Elution with (75-100 %) ethyl acetate/n-hexanes and further with (1-25%) methanol/ethyl acetate gave 46% (0.65 g) of pure yellow compound; mp 105-106 °C; IR (KBr) 3438, 2855, 2734, 2212, 1625, 1569, 1483, 1457, 1385, 1222, 1153; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 3.09-3.13 (m,4H), 7.49-7.66 (m,2H), 7.91 (d,1H,J=1.46); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 33.13,40.99, 106.27, 116.57, 121.04, 121.19, 127.08, 140.40, 142.59, 158.82; HRMS (EI) calculated for C<sub>10</sub>H<sub>10</sub>N<sub>4</sub> m/z 186.0905 found 186.0906.

b. **5-Cyano-2-(2-acetamidoethyl)benzimidazole.** 5-Cyano-2-(2-aminoethyl)benzimidazole (0.4 g, 2.15 mmol) was refluxed in dry THF (10 ml),

acetic anhydride (0.4 ml) and triethylamine (0.4 ml) for 4 hrs. The mixture was neutralized with 2N NaOH and extracted with ethyl acetate to give the crude acetamide. The acetamide was purified using flash column chromatography. Elution with (0-15 %) methanol/ethyl acetate gave 70% of pure white  
5 compound; mp 218-219 °C; IR (KBr) 3233, 3053, 2225, 1653, 1573, 1442, 1374, 1303, 1058, 815; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 1.78 (s,3H), 3.26 (t,2H), 3.53-3.62 (2H, J= 6.45, 12.38), 7.89-7.95 (dd, 1H, J=1.42, 8.48), 8.00 (d, J=8.22,1H), 8.19 (t,1H), 8.45 (d,1H, J=1.42); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 22.57, 27.82, 36.71, 108.06, 115.52, 118.62, 119.33, 129.02,  
10 131.29, 134.26, 156.23, 170.18; Anal. calculated for C<sub>12</sub>H<sub>12</sub>N<sub>4</sub>O.1/4H<sub>2</sub>O: C; 61.92, H; 5.41, N; 24.07 found C; 62.60, H; 5.41, N; 23.82.

c. **5-Formyl-2-(2-acetamidoethyl)benzimidazole.** 5-Cyano-2-(2-acetamidoethyl)benzimidazole (94.6 mg, 0.41 mmol) was refluxed in HCOOH  
15 (5.9 ml), water (0.5 ml) and Ni-Al (0.4 g) catalyst for six hours. The mixture was filtered hot through celite and the bed washed with methanol. The washings were concentrated *in vacuo*. The concentrate was basified with 2N NaOH to pH 9 and extracted with ethyl acetate. The ethyl acetate layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and purified by flash column chromatography. (0-15%)  
20 methanol/ethyl acetate gave 80% (70.10 mg) of pure white product; mp 220-221 °C; IR (KBr) 3222, 3047, 1656, 1575, 1439, 1289, 1110, 1058, 816; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 1.79 (s,3H), 3.29 (t, 2H), 3.54-3.64 (q, J= 6.66, 12.64, 2H), 7.97-8.09 (m,2H), 8.19 (t,1H), 8.38 (s, 1H), 10.17 (s,1H); <sup>13</sup>C (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 22.69, 27.91, 36.75, 114.89, 116.86, 125.84,  
25 131.67, 133.81, 135.33, 155.88, 170.13, 192.47; Anal. calculated for C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>: C; 62.32, H; 5.67, N; 18.17 found C; 62.10, H; 5.7, N; 17.92.

**Example 10. 5-Phenyl-2-[2'-(2-methoxyethyl)benzimidazol-5"-yl]benzimidazol-5'-yl]benzimidazole (13).**

30 5-Phenyl-2-[3,4-diaminophenyl]benzimidazole (0.17 mg, 0.58 mmol) and 5-formyl-2-(2-methoxyethyl)benzimidazole (120 mg, 0.59 mmol) were heated together in of nitrobenzene (5 ml) for 15 hours at 145 °C. Nitrobenzene was removed with a Kugelrohr and the compound was loaded onto

a column. (1-12%) methanol/ethyl acetate gave 80% pure yellowish product; mp >280 °C; IR (KBr) 3157, 2933, 1629, 1551, 1441, 1385, 1288, 1108, 818, 760, 698; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 3.33 (s,3H), 3.45 (t,2H), 3.89 (t,2H), 7.45-7.59 (m,3H), 7.79 (d,2H,J=7.04), 7.86-7.99 (m,2H), 8.06-8.14 (m,3H), 8.26-8.31 (dd,1H, J=1.34, 8.4), 8.44-8.49 (dd,1H,J=1.02,8.82), 8.72 (s,2H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 27.71, 58.30, 68.17, 111.7, 113.69, 114.57, 115.22, 115.72, 116.19, 118.51, 123.94, 124.21, 125.26, 125.61, 127.46, 128.14, 129.36, 131.65, 131.71, 132.91, 133.49, 137.24, 138.72, 139.68, 150.15, 153.24, 155.27; HRMS (FAB) calculated for C<sub>30</sub>H<sub>25</sub>N<sub>6</sub>O (MH<sup>+</sup>) 485.2089 found 485.2089.

The intermediate 5-formyl-2-(2-methoxyethyl)benzimidazole was prepared as follows.

a. **5-Cyano-2(2-methoxyethyl)benzimidazole.**

3,4-Diaminobenzonitrile (0.2 g, 1.5 mmol) was refluxed with (0.22 ml, 2.25 mmol) of 2-methoxypropionic acid in 4N HCl (2.5 ml) for 7 hours. After neutralization with 2N NaOH the mixture was extracted with ethyl acetate and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The product was purified on column chromatography using (7-100%) ethyl acetate/n-hexanes to give a 50 % yield of pure white product; mp 122-123 °C; IR (KBr) 2875, 2225, 1624, 1544, 1454, 1288, 1215, 1106, 824; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 3.29 (s,3H), 3.42 (t,2H), 3.84 (t,2H), 7.87-8.00 (m,2H), 8.40 (s,1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 27.65, 58.18, 68.02, 106.79, 115.56, 118.28, 119.37, 128.95, 131.31, 134.26, 156.12; Anal. calculated for C<sub>11</sub>H<sub>11</sub>N<sub>3</sub>.1/2 H<sub>2</sub>O: C; 62.84, H; 5.75, N; 19.97 found C; 62.71, H; 5.70, N; 18.83.

b. **5-Formyl-2-(2'-methoxyethyl)benzimidazole.** 5-Cyano-2-(2-

methoxyethyl)benzimidazole (0.12 g, 0.61 mmol) was mixed in HCOOH (9 ml) and water (1 ml), to which was added Ni-Al (0.6 g). The mixture was heated at 95 °C for 5.5 hours. The mixture was passed through a celite bed while hot and the bed was washed with methanol and the filtrate concentrated. After neutralization with 2N NaOH and extraction with CHCl<sub>3</sub>, the extracts were dried

over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The residue was purified by column chromatography using a gradient of 15-100% ethyl acetate/n-hexanes to give a white product in 65% yield; mp 83-84 °C; IR (Nujol) 2846, 2728, 1692, 1290, 1109, 816;  $^1\text{H}$  NMR ( $\text{DMSO-d}_6$  + 3 drops  $\text{CF}_3\text{COOH}$ )  $\delta$  3.31(s,3H), 3.44 (t,2H), 3.86 (t,2H), 7.79-8.09 (m,2H), 8.36 (s,1H), 10.16 (s,1H);  $^{13}\text{C}$  NMR ( $\text{DMSO-d}_6$  + 3 drops  $\text{CF}_3\text{COOH}$ )  $\delta$  27.73, 58.28, 68.08, 114.92, 116.86, 125.92, 131.55, 133.85, 135.19, 155.8, 192.43; Anal. calculated for  $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2 \cdot 1/4 \text{H}_2\text{O}$ : C; 64.69, H; 5.92, N; 13.71 found C; 64.76, H; 6.02, N; 13.60.

10 **Example 11. 5-Bromo-2-[2'-[2''-hydroxybenzimidazol-5''-yl]benzimidazol-5'-yl]benzimidazole (18).**

5-Bromo-2-[3,4-diaminophenyl]benzimidazole (33.3 mg, 0.11 mmol) and 5-formyl-2-hydroxybenzimidazole (17.7 mg, 0.11 mmol) were heated at 145 °C in nitrobenzene (3 ml) overnight. Nitrobenzene was removed with a Kugelrohr and the compound was purified by flash column chromatography. Elution with (5-15%) methanol/ethyl acetate gave 23.5 mg (0.05 mmol) of yellow colored compound in 48% yield; mp > 280 °C; IR (KBr) 3409, 3211, 1698, 1558, 1482, 1384, 1279;  $^1\text{H}$  NMR ( $\text{DMSO-d}_6$  + 3 drops  $\text{CF}_3\text{COOH}$ )  $\delta$  7.30 (d,1H,J=8.34), 7.69 (dd,1H,J=1.80, 8.71), 7.82 (s,1H), 7.86-7.99 (m,2H), 8.09-8.13 (m,2H), 8.32 (dd,1H,J=1.36,8.69), 8.61 (s,1H), 11.38 (s,1H);  $^{13}\text{C}$  ( $\text{DMSO-d}_6$  + 3 drops  $\text{CF}_3\text{COOH}$ )  $\delta$  108.01, 10.55, 113.92, 115.16, 115.27, 116.25, 117.19, 117.86, 121.06, 122.41, 125.24, 128.70, 130.78, 132.66, 133.16, 134.85, 134.89, 135.62, 150.42, 153.09, 155.69; HRMS (FAB) calculated for  $\text{C}_{21}\text{H}_{14}\text{BrN}_6\text{O}$  ( $\text{MH}^+$ ) 445.0412 found 445.0408.

25 The intermediate 5-bromo-2-[3,4-diaminophenyl]benzimidazole was prepared as follows.

a. **4-Bromo-*o*-phenylenediamine.** 4-Bromo-2-nitroaniline 600 mg (2.76 mmol) was dissolved in 25 ml absolute ethanol and 2.72 g (14 mmol)  $\text{SnCl}_2$  was added. The mixture was refluxed overnight. Ethanol was removed *in vacuo* and the mixture basified with 2N NaOH to pH 11. Ether extraction, drying the ether layer over anhydrous  $\text{Na}_2\text{SO}_4$  and concentration *in vacuo* afforded 486



mg (2.6 mmol, 94% yield) of the crude 4-bromo-*o*-phenylenediamine which was used for the next step without characterization.

b. **5-Bromo-2-[3,4-nitrophenyl]benzimidazole.** 4-Bromo-*o*-phenylenediamine (275 mg, 1.5 mmol) and 3,4-dinitrobenzaldehyde (300 mg, 1.5 mmol) in 2 ml nitrobenzene were heated at 145 °C overnight. The nitrobenzene was removed with a Kugelrohr. Column purification (1-10 % ethyl acetate/*n*-hexanes) afforded 209 mg (0.57 mmol, 39% yield) of the pure product; <sup>1</sup>H NMR (DMSO + 3 drops CF<sub>3</sub>COOH) δ 7.48 (dd, 1H, J=1.82, 8.76), 7.7 (d, 1H, 8.76), 7.96 (d, 1H, 1.46), 8.45 (d, 1H, 8.5), 8.68 (dd, 1H, J=1.84, 8.5), 8.93 (d, 1H, 1.84); <sup>13</sup>C NMR (DMSO + 3 drops CF<sub>3</sub>COOH) δ 116.36, 117.48, 118.66, 123.74, 127.05, 132.07, 134.49, 137.59, 140.23, 142.39, 142.81, 148.45.

c. **5-Bromo-2-[3,4-diaminophenyl]benzimidazole.** 5-Bromo-2-[3,4-dinitrophenyl]benzimidazole (140 mg, 0.38 mmol) was dissolved in 8 ml absolute ethanol. 0.8 g SnCl<sub>2</sub> (4.2 mmol) was added and the mixture refluxed overnight. Ethanol was removed *in vacuo* and the mixture basified with 2N NaOH to pH 11. Repeated extraction with ether, drying the ether layer over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentration *in vacuo* yielded (0.11 g, 0.37 mmol) of the crude diamine in 98% yield. The diamine was used without further purification; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 6.63 (d, 1H, J=8.1), 7.26 (m, 2H), 7.39 (m, 2H), 6.65 (d, 1H, J=1.76).

The intermediate 5-formyl-2-hydroxybenzimidazole was prepared as follows.

25

d. **5-Cyano-2-hydroxybenzimidazole.** 3,4-Diaminobenzonitrile (0.5 g, 3.8 mmol) and urea (0.25 g, 4.2 mmol) were heated together in 1 ml DMF at 145-150 °C for 9 hours. The DMF was concentrated *in vacuo* and the mixture was suspended in water and extracted with ethyl acetate. Purification by column chromatography (elution with 60/40 ethyl acetate/*n*-hexanes) provided (0.53 g, 3.3 mmol) a yield of 89% of the product; IR (KBr) 3533, 3209, 2224, 1732, 1482, 1281; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 7.1 (d, 1H, J=8.06),

30

7.09-7.41 (m,2H), 11.06 (s,1H), 11.19 (s,1H);  $^{13}\text{C}$  NMR (DMSO- $d_6$  + 3 drops  $\text{CF}_3\text{COOH}$ )  $\delta$  102.52, 109.36, 111.61, 120.04, 126.03, 130.18, 133.93, 155.41.

e. **5-Formyl-2-hydroxybenzimidazole. 5-Cyano-2-**

- 5 hydroxybenzimidazole (0.5 g, 3.14 mmol) was mixed with Ni-Al catalyst (3 g),  $\text{HCOOH}$  (45 ml) and water (3 ml) and heated to reflux for 12 hrs. The hot mixture was filtered through a bed of celite and the filtrate concentrated *in vacuo*. pH of the mixture was adjusted to 9 with 2N NaOH and extraction was carried out with ethyl acetate. The ethyl acetate layer was dried over anhydrous
- 10  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. Column chromatographic purification, and elution with 65/35 ethyl acetate/n-hexanes provided 0.4 g (2.4 mmol, 76% yield) of the final compound; IR (KBr) 3261, 3160, 1677, 1631, 1477, 1282, 711;  $^1\text{H}$  NMR (DMSO- $d_6$  + 3 drops  $\text{CF}_3\text{COOH}$ )  $\delta$  7.11 (d,1H,J=8.0), 7.41 (s,1H), 7.57 (dd,1H,J=1.54,8.0), 9.86 (s,1H), 10.99 (s,1H), 11.17 (s,1H);  $^{13}\text{C}$  NMR (DMSO-
- 15  $d_6$  + 3 drops  $\text{CF}_3\text{COOH}$ )  $\delta$  108.15, 108.67, 125.37, 130.1, 130.42, 135.64, 155.68, 191.99.

**Example 12. 5,6-Dibromo-2-[2'-(2"-trifluoromethylbenzimidazol-5"-yl)benzimidazol-5'-yl]benzimidazole (22).**

- 20 4,5-Dibromo-*o*-phenylenediamine (60 mg, 0.22 mmol) and 5-formyl-2-(2'-trifluoromethylbenzimidazol-5'-yl)benzimidazole (73 mg, 0.22 mmol) were heated in 10 ml nitrobenzene overnight at 150 °C. The nitrobenzene was removed with a Kugelrohr and the mixture loaded on a column. Elution with 90/10 ethyl acetate/n-hexanes yielded 25 mg (20% yield, 0.05 mmol) of
- 25 compound; mp > 260 °C;  $^1\text{H}$  NMR (DMSO- $d_6$  + 3 drops  $\text{CF}_3\text{COOH}$ )  $\delta$  8.02-8.09 (m,2H), 8.14 (s,2H), 8.26-8.29 (m,2H), 8.57 (s,1H), 8.69 (s,1H).

The intermediate 5-formyl-2-(2'-trifluoromethylbenzimidazol-5'-yl)benzimidazole was prepared as follows.

- 30 a. **5-Cyano-2-(2'-trifluoromethylbenzimidazol-5'-yl)benzimidazole.** 5-formyl-2-trifluoromethylbenzimidazole (1.5 g; 7 mmol) was mixed with 0.93 g of 3,4-diaminobenzonitrile (7 mmol) and 15 ml nitrobenzene. The mixture was heated overnight at 145 °C. Nitrobenzene was

removed with a Kugelrohr and the mixture was loaded onto a column. Elution with 40/60 ethyl acetate/n-hexanes gave 0.9 g (2.75 mmol) of the compound in 40% yield; mp > 260 °C; IR (KBr) 3254, 2916, 2223, 1614, 1175; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 7.74 (dd, 1H, J=1.38, 8.4), 7.88 (d, 1H, J=8.14), 7.98 (d, 1H, J=8.78), 8.25-8.03 (m, 2H), 8.63 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 105.62, 115.78, 116.35, 120.26, 123.06, 123.82, 127.18, 137.35, 139.65, 142.17, 142.96, 154.13

b. **5-Formyl-2-(2'-trifluoromethylbenzimidazol-5'-yl)benzimidazole.** 5-Cyano-2-(2'-trifluoromethylbenzimidazol-5'-yl)benzimidazole (0.18 g, 0.54 mmol) was refluxed in 8 ml HCOOH with 2.5 ml water and 0.6 g Ni-Al catalyst for 7 hours at 110 °C. The hot mixture was filtered through celite bed and the filtrate concentrated and its pH adjusted to 9.0 with 2N NaOH. Repeated extraction with ethyl acetate, drying the organic layer with anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentration *in vacuo* gave the crude product. Purification by column chromatography (elution with 1% methanol/ethyl acetate) yielded 87 mg (0.26 mmol, 49% yield) of the pure aldehyde; mp > 260 °C; IR (KBr) 3411, 2966, 1679, 1310, 1153; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 7.96-8.06 (m, 3H), 8.30-8.32 (m, 2H), 8.57 (s, 1H), 10.15 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 106.77, 112.51, 113.28, 117.79, 117.84, 118.28, 121.78, 123.96, 125.02, 132.98, 135.79, 139.62, 142.48, 153.51, 192.65.

The intermediate 4,5-dibromo-1,2-phenylenediamine was prepared as follows, using a modification of the procedures described in U.S. Patent Number 4,264,600.

c. **3,4-Dibromoaniline.** 3-Bromoaniline (3 g, 17.4 mmol) was dissolved in 15 ml CH<sub>2</sub>Cl<sub>2</sub> and the reaction mixture was cooled to -10 °C in an ice-salt bath. 2,4,4,6-Tetrabromo-2,5-cyclohexadienone, (9.29 g, 0.02 mmol) was added in small portions with constant stirring. The reaction mixture was stirred for a period of 7 hours at 0 °C after which the reaction was quenched with 2N NaOH (10 ml). The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> and the organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The CH<sub>2</sub>Cl<sub>2</sub> layer was concentrated *in*

*vacuo* and purified by column chromatography. Elution with 0-3% ethyl acetate/n-hexanes yielded 3.2 g (12.7 mmol, 73%) of pure product; mp 80-81 °C; IR (KBr) 3406, 3318, 3210, 1583, 1464, 1287, 1108, 860, 668; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.49 (dd, 1H, J=2.7, 8.6), 6.7 (d, 1H, J=2.64), 7.32 (d, 1H, J=8.6); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 112.6, 116, 120.1, 125.45, 134.26, 147.08.

d. **3,4-Dibromoacetanilide.** 3,4-Dibromoaniline (3.2 g, 12.7 mmol) was mixed with 13 ml acetic anhydride and heated at 110 °C for 2 hours after which it was poured over ice cold water. The aqueous layer was extracted with ethyl acetate, 0.1N HCl and 1N NaOH, and the organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The organic layer was concentrated *in vacuo* and purified by column chromatography. Elution with 1-50 % ethyl acetate/n-hexanes yielded 2.6 g (9 mmol, 71% yield) of the acetanilide: mp 91-93 °C; IR (KBr) 3293, 3172, 1668, 1589, 1552, 1522, 1373, 813; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.17 (s, 3H), 7.33 (dd, 1H, J=2.4, 8.66), 7.52 (d, 1H, J=8.68), 7.87 (d, 1H, J=2.4); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 25.07, 119.64, 120.35, 125.04, 125.38, 134.13, 138.40, 168.96.

e. **3,4-Dibromo-6-nitroacetanilide.** 3,4-Dibromoacetanilide (2.64 g, 9 mmol) was dissolved in 8 ml conc. H<sub>2</sub>SO<sub>4</sub> (chilled in ice-salt bath) at a temperature of 0 °C. To this solution was added 1 ml chilled nitrating mixture (equal volumes of conc. H<sub>2</sub>SO<sub>4</sub> and fuming HNO<sub>3</sub>) and the mixture stirred for 20 min. The reaction mixture was poured into ice water and neutralized with ammonium hydroxide to pH of 9.0. The yellow precipitate was filtered and purified by column chromatography. Elution with 1-4 % ethyl acetate/n-hexanes yielded 2.1 g (6.15 mmol, 68%) of the 3,4-dibromo-6-nitroacetanilide: mp 141-142 °C; IR (KBr) 3340, 3128, 1693, 1569, 1479, 1332, 1268; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.3 (s, 3H), 8.46 (s, 1H), 9.22 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 26.14, 118.67, 126.86, 130.86, 134.51, 134.59, 136.98, 169.40.

f. **3,4-Dibromo-6-nitroaniline.** 3,4-Dibromo-6-nitroacetanilide (2.1 g, 6.15 mmol) was heated to refluxing temperature for 2 hours with 40 ml of 6N HCl. The reaction mixture was then poured over an ice-water mixture and stirred. The pH of the solution was adjusted to 9.0 with NaOH. The resulting

bright yellow precipitate was filtered, washed with cold water and dried to yield (1.78 g, 6 mmol, 98%) of pure product; mp 204-205 °C; IR (KBr) 3475, 3355, 3097, 1612, 1478, 1241, 1122, 914; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.05 (brs, 2H), 7.18 (s, 1H), 8.37 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 111.23, 123.22, 129.34, 130.59, 133.61, 142.36.

g. **4,5-Dibromo-1,2-phenylenediamine.** 3,4-Dibromo-6-nitroaniline (189 mg, 0.64 mmol) was dissolved in 8 ml anhydrous ethanol to which was added about 400 mg Raney Nickel. The hydrogenation apparatus was at a hydrogen pressure 50 psi. After 45 min. the deep yellow color originally present was completely discharged indicating complete reduction of the nitro to the amine groups. The reaction mixture was filtered through celite and the bed was washed with methanol. The methanol was concentrated *in vacuo* to give 135 mg of the crude diamine in 82% yield. The crude diamine was used as such without further purification; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.37 (br, 4H), 6.93 (s, 2H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 113.98, 120.95, 135.84.

**Example 13. 5-Phenyl-6-methoxy-2-[2'-(benzimidazol-5"-yl)benzimidazol-5'-yl]benzimidazole (15).**

4-Methoxy-5-phenyl-1,2 phenylenediamine (139 mg, 0.65 mmol) and 5-formyl-2-(benzimidazol-5'-yl)benzimidazole (170 mg, 0.65 mmol) were heated together in nitrobenzene (5 ml) overnight at 145 °C. Nitrobenzene was removed with a Kugelrohr and the compound was loaded onto a column. (1-10%) methanol/ethyl acetate gave 120 mg (41%) of pure product; mp > 280 °C; IR (KBr) 3298, 3050, 2987, 1630, 1541, 1438, 1283; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 3.92 (s, 3H), 7.39-7.57 (m, 6H), 7.72 (s, 1H), 8.11-8.15 (m, 1H), 8.19-8.27 (m, 2H), 8.47 (dd, 1H, J=1.46, 8.07), 8.64 (s, 1H), 8.76 (s, 1H), 9.75 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 56.40, 95.73, 114.62, 115.18, 116.02, 116.15, 119.03, 119.07, 125.59, 126.29, 127.63, 127.67, 128.34, 129.79, 130.69, 131.54, 132.42, 133.38, 137.59, 148.81, 152.84, 156.82; HRMS (FAB) calculated for C<sub>28</sub>H<sub>21</sub>N<sub>6</sub>O (MH<sup>+</sup>) 457.1777, found 457.1770.

The intermediate 4-methoxy-5-phenyl-1,2 phenylenediamine was prepared as follows.

- a. **4-Methoxy-2-nitro-5-phenylaniline.** 3-Bromo-4-methoxy-6-nitroaniline (400 mg, 1.62 mmol) was dissolved in DME (20 ml). Tetrakis(palladium triphenyl phosphine (94 mg, 0.08mmol) served as the catalyst. To this mixture was added phenyl boronic acid (300 mg, 1.01 mmol) and 2M Na<sub>2</sub>CO<sub>3</sub> (1.8 ml) and the mixture refluxed at 90 °C overnight. The reaction mixture was concentrated *in vacuo* and purified by flash column chromatography. (1-10%) ethyl acetate/n-hexanes gave 280 mg (1.14 mmol) of pure product in 71% yield; IR (KBr) 3435, 3328, 2933, 1570, 1480, 1227, 1029, 692; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.80 (s,3H), 5.94 (br,2H), 6.79 (s,1H), 7.39-7.64 (m,5H), 7.64 (s,1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 56.63, 106.76, 121.26, 128.69, 128.72, 128.76, 128.79, 128.92, 129.71, 136.69, 140.24, 141.42, 148.65; MS (FAB) calculated for C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub> 244.0848, found 244.0847.
- b. **4-Methoxy-5-phenyl-1,2 phenylenediamine.** 4-Methoxy-2-nitro-5-phenyl-aniline (165 mg, 0.68 mmol) in ethyl acetate (20 ml) was reduced using 20 mg of 10% Pd/C as the catalyst, overnight. The mixture was filtered through celite bed and the bed was washed with methanol. All the washings were concentrated *in vacuo* to yield 139 mg (96% yield) of the crude diamine which was used without further purification; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.49 (brs,4H), 3.71 (s,3H), 6.42 (s,1H), 6.74 (s,1H), 7.26-7.52 (m,5H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 56.81, 101.77, 120.93, 127.53, 128.39, 128.45, 128.49, 129.82, 129.96, 136.64, 139.17, 142.37, 153.73.

The intermediate 3-bromo-4-methoxy-6-nitro-aniline used in sub-part a is commercially available (Aldrich Chemical Company).

The intermediate 5-formyl-2-(benzimidazol-5'-yl)benzimidazole was prepared as described by Sun et al. *J. Med. Chem.* **1995**, *38*, 3638-3644.

**Example 14. 5,6-Diphenyl-2-[2'-(benzimidazol-5"-yl)benzimidazol-5'-yl]benzimidazole (16).**

4,5-Diphenyl-1,2-phenylenediamine (151 mg, 0.58 mmol) and 5-formyl-2-(benzimidazol-5'-yl)benzimidazole (152 mg, 0.58 mmol) were heated  
5 in nitrobenzene (4 ml) overnight at 145 °C under nitrogen. The nitrobenzene was removed with a Kugelrohr. Compound was purified by flash column chromatography. Elution with (2-10%) methanol/ethyl acetate gave 108 mg (0.22 mmol) of pure yellow compound in 37% yield. mp > 280 °C; IR (KBr) 3399, 3059, 1629, 1551, 1441, 1292; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH)  
10 δ 7.17-7.33 (m, 10H), 7.84 (s, 2H), 8.06-8.25 (m, 3H), 8.49 (dd, 1H, J=1.18, 8.89), 8.66 (s, 1H), 8.75 (s, 1H), 9.69 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 113.94, 115.24, 115.84, 116.15, 116.32, 117.89, 123.34, 126.32, 126.66, 127.86, 128.38, 128.42, 128.46, 130.05, 131.77, 131.89, 133.03, 138.87, 138.97, 140.62, 141.19, 160.97, 153.71; HRMS (FAB) calculated for C<sub>33</sub>H<sub>23</sub>N<sub>6</sub> (MH<sup>+</sup>)  
15 503.1984 found 503.1989.

The intermediate 4,5-diphenyl-1,2-phenylenediamine was prepared as follows.

a. **4,5-Diphenyl-2-nitroaniline.** 3,4-Dibromo-6-nitroaniline (332  
20 mg, 1.12 mmol) was dissolved in DME (20 ml). Tetrakis(triphenylphosphine)palladium (65 mg, 0.06 mmol), phenyl boronic acid (200 mg, 1.64 mmol), and 2M Na<sub>2</sub>CO<sub>3</sub> (10 ml) were added to the reaction mixture and refluxed at 90 °C overnight. The reaction mixture was concentrated  
in *vacuo* and loaded onto a column. (1-3%) ethyl acetate/n-hexanes afforded 259  
25 mg of pure yellow compound in 80% yield; mp 139-141° C; IR (KBr) 3476, 3363, 2924, 1621, 1476, 1263, 1089; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.11 (brs, 2H), 6.86 (s, 1H), 7.04-7.09 (m, 5H), 7.15-7.26 (m, 5H), 8.21 (s, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 120.56, 127.22, 127.66, 128.20, 128.26, 128.52, 128.53, 129.31, 129.39, 129.47, 129.77, 130.14, 130.93, 131.79, 139.85, 139.94, 144.08, 149.03.

- b. **4,5-Diphenyl-1,2-phenylenediamine.** 4,5-Diphenyl-2-nitroaniline (200 mg, 0.69 mmol) in ethanol (50 ml) was reduced using 40 mg 10% Pd/C. Hydrogenation was carried out at 40 psi pressure for 10 hours. The mixture was filtered through celite and the bed washed with methanol. The methanol layer was concentrated *in vacuo* to yield 151 mg of the crude diamine in 84% yield. The crude diamine was used as such without purification. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 6.78 (s, 2H), 7.01-7.16 (m, 10H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 118.08, 121.23, 127.17, 127.66, 130.44, 131.76, 138.32.

10 **Example 15. 5,6-Dibromo-2-[2'-(benzimidazol-5''-yl)benzimidazol-5'-yl]benzimidazole (20).**

- 4,5-Dibromo-1,2-phenylenediamine (128 mg, 0.48 mmol) and 5-formyl-2-(benzimidazol-5'-yl)benzimidazole (126 mg, 0.48 mmol) were heated in nitrobenzene (6 ml) at 145 °C overnight. The nitrobenzene was removed with a Kugelrohr and the mixture loaded on a column for purification. Elution with (1-10%) methanol/ethyl acetate gave 0.1 gm (41 %) of pure compound; mp > 260° C; IR (KBr) 3405, 3198, 1626, 1544, 1385, 1292; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 8.03-8.13 (m, 2H), 8.17 (s, 2H), 8.25 (d, 1H, J=9.16), 8.42 (d, 1H, J=8.64), 8.59 (s, 1H), 8.74 (s, 1H), 9.75 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 114.73, 114.96, 115.77, 115.99, 118.93, 119.61, 120.73, 123.29, 124.47, 125.56, 131.49, 133.47, 134.77, 135.79, 137.95, 151.93, 152.31; HRMS (FAB) calculated for C<sub>21</sub>H<sub>13</sub>Br<sub>2</sub>N<sub>6</sub> (MH<sup>+</sup>) 506.9568 found 506.9574.

The intermediate 4,5-dibromo-1,2-phenylenediamine was prepared as described at Example 12c-g.

25

**Example 16. 5-Bromo-6-methoxy-2-[2'-(benzimidazol-5''-yl)benzimidazol-5'-yl] benzimidazole (21).**

- In nitrobenzene (4ml), 4-bromo-5-methoxy-1,2-phenylenediamine (123 mg, 0.57 mmol) and 5-formyl-2-(benzimidazol-5'-yl)benzimidazole (150 mg, 0.57 mmol) were heated at 145 °C overnight under

30



nitrogen. Nitrobenzene was removed with a Kugelrohr. Chromatographic separation (column) with (1-10%) methanol/ethyl acetate afforded 104 mg (40%) of the title compound; mp > 280° C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 3.91 (s,3H), 7.17-7.29 (m,1H), 7.78 (d,1H,J=8.84), 8.00-8.13 (m,3H), 8.49-8.53 (m,1H), 8.66 (s,1H), 8.79 (s,1H), 9.69 (s,1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH) δ 56.16, 96.43, 114.06, 114.97, 115.55, 115.79, 115.89, 116.35, 117.97, 118.42, 123.11, 125.30, 125.58, 126.33, 127.43, 131.85, 133.05, 133.11, 138.39, 140.84, 149.04, 153.44; HRMS (FAB) calculated for C<sub>22</sub>H<sub>16</sub>BrN<sub>6</sub>O (MH<sup>+</sup>) The intermediate 4-bromo-5-methoxy-1,2-phenylenediamine was prepared as follows.

a. **4-Bromo-5-methoxy-1,2-phenylenediamine.** 3-Bromo-4-methoxy-6-nitroaniline (150 mg, 0.61 mmol) (Aldrich Chemical Company) was dissolved in ethanol (10 ml) and hydrogenation was carried out using 350 mg Raney Nickel for 30 min. The reaction mixture was filtered through celite bed and washed with methanol. The methanolic layer was dried *in vacuo* to give 127 mg of the crude diamine in 97.40% yield, which was used as such without purification; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.20 (brs,2H), 3.39 (brs,2H), 3.8 (s,3H), 8.36 (s,1H), 6.89 (s,1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 57.35, 100.38, 102.27, 122.52, 128.69, 136.68, 150.94.

**Example 17. 5-(4-Chlorophenyl)-2-[2'-(2''-trifluoromethylbenzimidazol-5''-yl)]benzimidazol-5'-yl]benzimidazole (23).**

2-(3,4-Diaminophenyl)-5-(4-chlorophenyl)benzimidazole (65 mg) and 5-formyl-2-trifluoromethylbenzimidazole (43 mg) were heated together in nitrobenzene (4 mL) at 150 °C. The nitrobenzene was removed with a Kugelrohr, and the crude mixture was loaded on a column. Elution with (10-90%) ethyl acetate/hexanes gave (32 mg, 0.006 mmol) of the title compound, 30% yield; mp > 260 °C; IR (KBr) 3153, 2965, 1546, 1444, 1162, 810; UV (MeOH) 335, 240 nm (log ε = 4.45, 4.40); <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops

CF<sub>3</sub>COOH)  $\delta$  7.56-7.64 (m, 2H), 7.73-8.06 (m, 6H), 8.17-8.25 (m, 2H), 8.33 (dd, 1H, J = 1.46, 8.81), 8.67 (d, 1H, J = 1.40); <sup>13</sup>C NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH)  $\delta$  114.76, 115.49, 116.00, 117.32, 117.45, 123.28, 123.89, 124.11, 125.13, 125.19, 127.45, 127.49, 129.25, 129.36, 129.42, 132.49, 133.06, 133.53, 136.94, 137.04, 138.23, 138.62, 140.56, 150.64, 150.84, 154.37; HRMS (FAB) calculated for C<sub>28</sub>H<sub>17</sub>ClF<sub>3</sub>N<sub>6</sub> (MH<sup>+</sup>) 529.1155 found 529.1156.

The intermediate 2-(3,4-diaminophenyl)-5-(4-chlorophenyl)-benzimidazole was prepared as follows.

10 a. **4-(4-Chlorophenyl)-1,2-phenylenediamine.** 4-(4-chlorophenyl)-2-nitroaniline (0.79 g) in a mixture of methanol:ethyl acetate (1:1, 40 mL) was reduced using 115 mg of 10% Pd/C. Hydrogenation was carried out at 40 psi for 10 hours. The mixture was filtered through celite and the bed washed with methanol. The methanol layer was condensed under vacuum to yield 0.71 g of the  
15 diamine, which was used without further purification.

b. **2-(3,4-Dinitrophenyl)-5-(4-chlorophenyl)benzimidazole.** A stirred solution of 4-(4-Chlorophenyl)-1,2-phenylenediamine (0.7 g) and 3,4-dinitrobenzaldehyde (0.63 g) in nitrobenzene (13 mL) was heated at 150 °C  
20 under nitrogen for 16 hours. The solvent was removed with a Kugelrohr and the mixture was purified by flash chromatography. Elution with ethyl acetate:hexanes (1-10%) provided the benzimidazole as a bright yellow-red compound (0.55 g, 44%); mp 75-76 °C.

25 c. **2-(3,4-Diaminophenyl)-5-(4-chlorophenyl)benzimidazole.** 2-(3,4-Dinitrophenyl)-5-(4-chlorophenyl)benzimidazole (125 mg) was dissolved in ethyl acetate (25 mL) and reduced by hydrogenation using Pd/C 10% (25 mg) as the catalyst for 12 hours. Filtration through celite followed by evaporation of the solvent gave the diamine; <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + 3 drops CF<sub>3</sub>COOH)  $\delta$  6.92  
30 (d, 1H, J=8.42), 7.57-7.62 (m, 4H), 7.65-7.81 (m, 4H), 7.92 (s, 1H); <sup>13</sup>C NMR

(DMSO-d<sub>6</sub> + 3drops CF<sub>3</sub>COOH)  $\delta$  110.15, 111.28, 114.09, 115.29, 117.73, 128.18, 129.22, 129.33, 129.34, 131.87, 132.89, 132.97, 136.69, 138.68, 143.70, 150.78.

- 5 **Example 18.** The following illustrate representative pharmaceutical dosage forms, containing a compound of formula I ('Compound X'), for therapeutic or prophylactic use in humans.

<u>(i) Tablet 1</u>		<u>mg/tablet</u>
10	'Compound X'	100.0
	Lactose	77.5
	Povidone	15.0
	Croscarmellose sodium	12.0
	Microcrystalline cellulose	92.5
15	Magnesium stearate	3.0
		300.0

<u>(ii) Tablet 2</u>		<u>mg/tablet</u>
	'Compound X'	20.0
20	Microcrystalline cellulose	410.0
	Starch	50.0
	Sodium starch glycolate	15.0
	Magnesium stearate	5.0
		500.0

25	<u>(iii) Capsule</u>	<u>mg/capsule</u>
	'Compound X'	10.0
	Colloidal silicon dioxide	1.5
	Lactose	465.5
30	Pregelatinized starch	120.0
	Magnesium stearate	3.0
		600.0

42

	(iv) <u>Injection 1 (1 mg/ml)</u>	mg/ml
	'Compound X' (free acid form)	1.0
	Dibasic sodium phosphate	12.0
	Monobasic sodium phosphate	0.7
5	Sodium chloride	4.5
	1.0 N Sodium hydroxide solution	
	(pH adjustment to 7.0-7.5)	q.s.
	Water for injection	q.s. ad 1 mL
10	(v) <u>Injection 2 (10 mg/ml)</u>	mg/ml
	'Compound X' (free acid form)	10.0
	Monobasic sodium phosphate	0.3
	Dibasic sodium phosphate	1.1
	Polyethylene glycol 400	200.0
15	01 N Sodium hydroxide solution	
	(pH adjustment to 7.0-7.5)	q.s.
	Water for injection	q.s. ad 1 mL
	(vi) <u>Aerosol</u>	mg/can
20	'Compound X'	20.0
	Oleic acid	10.0
	Trichloromonofluoromethane	5,000.0
	Dichlorodifluoromethane	10,000.0
	Dichlorotetrafluoroethane	5,000.0

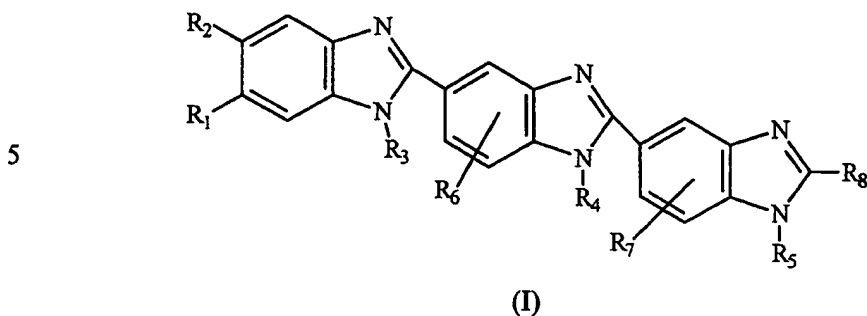
25

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art.

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

What is claimed is:

1. A compound of formula I:



wherein:

$R_1$  and  $R_2$  are each independently hydrogen,  $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio,  $(C_1-C_6)$ alkanoyloxy, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, or heteroaryl $(C_1-C_6)$ alkyl; or  $R_1$  and  $R_2$  taken together are methylenedioxy; or  $R_1$  and  $R_2$  taken together are benzo;

$R_3$ ,  $R_4$ , and  $R_5$  are each independently selected from the group consisting of hydrogen,  $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_2-C_6)$ alkanoyloxy, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, and heteroaryl $(C_1-C_6)$ alkyl;

$R_6$  and  $R_7$  are each independently hydrogen,  $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, or  $(C_1-C_6)$ alkanoyloxy;

$R_8$  is hydroxy, halo, nitro, cyano, mercapto, carboxy,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy,  $-NR_aR_b$ , halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl, halo $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio,  $(C_1-C_6)$ alkanoyloxy, aryloxy, heteroaryloxy,  $SR_c$ , or  $S(O)_nR_d$ ;

or  $R_8$  is  $(C_1-C_6)$ alkyl substituted by 1, 2, or 3 substituents independently selected from the group consisting of hydroxy, nitro, cyano, mercapto, carboxy,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy,  $-NR_aR_b$ , trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio,  $(C_1-C_6)$ alkanoyloxy, 5 aryloxy, and heteroaryloxy;

$n$  is 1 or 2;

each of  $R_a$  and  $R_b$  is independently hydrogen,  $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, heteroaryl $(C_1-C_6)$ alkyl, arylcarbonyl, or heteroarylcarbonyl; or  $R_a$  and  $R_b$  10 together with the nitrogen to which they are attached are pyrrolidino, piperidino, or morpholino;

$R_c$  is  $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, heteroaryl $(C_1-C_6)$ alkyl, arylcarbonyl, halo $(C_1-C_6)$ alkyl, or heteroarylcarbonyl;

15  $R_d$  is  $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, heteroaryl $(C_1-C_6)$ alkyl, arylcarbonyl, halo $(C_1-C_6)$ alkyl,  $NR_eR_f$ , or heteroarylcarbonyl; and

$R_e$  and  $R_f$  are each independently hydrogen,  $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkanoyl, aryl, heteroaryl, aryl $(C_1-C_6)$ alkyl, heteroaryl $(C_1-C_6)$ alkyl, 20 arylcarbonyl, halo $(C_1-C_6)$ alkyl, or heteroarylcarbonyl;

wherein any aryl, heteroaryl, or benzo of  $R_1-R_5$ ,  $R_8$ , and  $R_a-R_f$  may optionally be substituted by 1, 2, or 3 substituents independently selected from the group consisting of  $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy, 25 hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, and  $(C_1-C_6)$ alkanoyloxy;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein  $R_2$  is phenyl, optionally substituted by 1, 2, or 3 substituents independently selected from the group consisting of  $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl, 5  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, and  $(C_1-C_6)$ alkanoyloxy.
3. The compound of claim 1 wherein  $R_2$  is phenyl.
4. The compound of any claim 1 wherein  $R_1$  is hydrogen.
- 10 5. The compound of claim 1 wherein  $R_1$  is halo.
6. The compound of claim 1 wherein  $R_1$  and  $R_2$  are each halo.
- 15 7. The compound of claim 1 wherein  $R_1$  and  $R_2$  are each bromo.
8. The compound of claim 1 wherein  $R_3$ ,  $R_4$ , and  $R_5$  are each hydrogen.
- 20 9. The compound of claim 1 wherein  $R_6$  and  $R_7$  are each hydrogen.
10. The compound of claim 1 wherein  $R_8$  is  $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, or 25  $(C_1-C_6)$ alkanoyloxy.

11. The compound of claim 1 wherein  $R_7$  is  $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, or  $(C_1-C_6)$ alkanoyloxy.

5

12. The compound of any one of claims 1 to 11 wherein  $R_8$  is hydroxy, halo, nitro, cyano, mercapto, carboxy,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy,  $-NR_aR_b$ , halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio,  $(C_1-C_6)$ alkanoyloxy, aryloxy, or  
10 heteroaryloxy; or  $R_8$  is  $(C_1-C_6)$ alkyl substituted by 1, 2, or 3 substituents independently selected from the group consisting of hydroxy, nitro, cyano, mercapto, carboxy,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy,  $-NR_aR_b$ , trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio,  $(C_1-C_6)$ alkanoyloxy, aryloxy, and heteroaryloxy

15

13. The compound of any one of claims 1 to 11 wherein  $R_8$  is hydroxy, halo, nitro, cyano, mercapto, carboxy,  $(C_1-C_6)$ alkoxy,  $-NR_aR_b$ , halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, or  $(C_1-C_6)$ alkanoyloxy.

20

14. The compound of any one of claims 1 to 11 wherein  $R_8$  is  $(C_1-C_6)$ alkyl substituted by 1, 2, or 3 hydroxy, nitro, cyano, mercapto, carboxy,  $(C_1-C_6)$ alkoxy,  $-NR_aR_b$ , trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, or  $(C_1-C_6)$ alkanoyloxy.

25

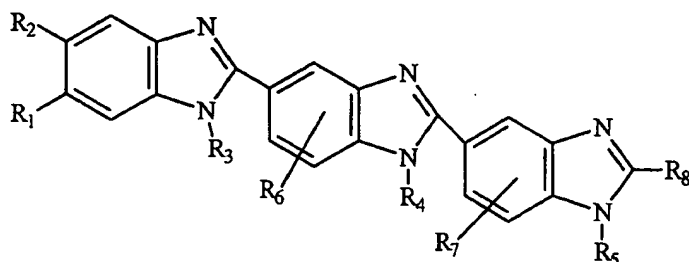
15. The compound of any one of claims 1 to 11 wherein  $R_8$  is  $(C_1-C_6)$ alkyl substituted by hydroxy, nitro, cyano, mercapto, carboxy,  $(C_1-C_6)$ alkoxy,  $-NR_aR_b$ , trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, or  $(C_1-C_6)$ alkanoyloxy.



16. The compound of any one of claims 1 to 11 wherein  $R_8$  is trifluoromethyl, chlorodifluoromethyl, dichlorofluoromethyl, 2,2,2-trifluoroethyl, perfluoroethyl, trifluoromethoxy, cyano, halo, carboxy,  $(C_1-C_6)$ alkoxycarbonyl, halo $(C_1-C_6)$ alkanoyl, or  $S(O)_nR_4$ .
- 5
17. The compound of any one of claims 1 to 11 wherein  $R_8$  is halo or trifluoromethyl.
18. The compound of any one of claims 1 to 11 wherein  $R_8$  is
- 10 trifluoromethyl.
19. The compound 5-phenyl-2-[2'-(2"-mercaptobenzimidazol-5"-yl)benzimidazol-5'-yl] benzimidazole; 5-phenyl-2-[2'(2"-trifluoromethylbenzimidazol-5"-yl) benzimidazol-5'-yl]benzimidazole; or 5,6-
- 15 dibromo-2-[2'-(2"-trifluoromethyl-benzimidazol-5"-yl)benzimidazol-5'-yl]benzimidazole; or a pharmaceutically acceptable salt thereof.
20. The compound 5-phenyl-2-[2'(2"-trifluoromethylbenzimidazol-5"-yl) benzimidazol-5'-yl]benzimidazole; or a pharmaceutically acceptable salt thereof.
- 20
21. A pharmaceutical composition comprising a compound of any one of claims 1 to 20, in combination with a pharmaceutically acceptable carrier.
22. A therapeutic method comprising treating cancer by administering to a
- 25 mammal in need of such therapy, an effective amount of a compound of any one of claims 1 to 20.

23. A method comprising inhibiting cancer cells by contacting said cancer cells with an effective amount of a compound of any one of claims 1 to 20.

24. A compound of formula I:



(I)

5 wherein:

$R_1$  and  $R_2$  are each independently (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy(C<sub>1</sub>-C<sub>6</sub>)alkyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy, aryl, heteroaryl, aryl(C<sub>1</sub>-C<sub>6</sub>)alkyl, or heteroaryl(C<sub>1</sub>-C<sub>6</sub>)alkyl; or  $R_1$  and  $R_2$  taken together are methylenedioxy;

$R_3$ ,  $R_4$ , and  $R_5$  are each independently selected from the group consisting of hydrogen, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>2</sub>-C<sub>6</sub>)alkanoyloxy, aryl, heteroaryl, aryl(C<sub>1</sub>-C<sub>6</sub>)alkyl, and heteroaryl(C<sub>1</sub>-C<sub>6</sub>)alkyl;

$R_6$  and  $R_7$  are each independently hydrogen, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>6</sub>)cycloalkyl(C<sub>1</sub>-C<sub>6</sub>)alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy(C<sub>1</sub>-C<sub>6</sub>)alkyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)alkanoyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, (C<sub>1</sub>-C<sub>6</sub>)alkylthio, or (C<sub>1</sub>-C<sub>6</sub>)alkanoyloxy; and

$R_8$  is hydrogen, (C<sub>1</sub>-C<sub>6</sub>)alkyl, aryl, or heteroaryl;

wherein any aryl or heteroaryl of  $R_1$ - $R_5$  and  $R_8$  may optionally be substituted by 1, 2, or 3 substituents independently selected from the group consisting of  $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, and  $(C_1-C_6)$ alkanoyloxy;

or a pharmaceutically acceptable salt thereof.

10 25. The compound of claim 24 wherein  $R_1$  and  $R_2$  are each independently  $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy, or aryl.

15 26. The compound of claim 24 wherein  $R_1$  and  $R_2$  taken together are methylenedioxy.

27. The compound of claim 24 wherein  $R_1$  and  $R_2$  are each halo.

28. The compound of claim 24 wherein  $R_1$  and  $R_2$  are each bromo.

20

29. The compound of claim 24 wherein  $R_1$  and  $R_2$  are each independently phenyl or methoxy.

30. The compound of claim 24 wherein  $R_3$ ,  $R_4$ , and  $R_5$  are each hydrogen.

25

31. The compound of claim 24 wherein  $R_6$  is  $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, or  $(C_1-C_6)$ alkanoyloxy.

5

32. The compound of claim 24 wherein  $R_7$  is  $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, or  $(C_1-C_6)$ alkanoyloxy.

10

33. The compound of claim 24 wherein  $R_8$  is hydrogen.

34. The compound of claim 24 wherein  $R_8$  is  $(C_1-C_6)$ alkyl.

15 35. The compound of claim 24 wherein  $R_8$  is aryl, optionally substituted by 1, 2, or 3 substituents independently selected from the group consisting of  $(C_3-C_6)$ cycloalkyl,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, and  $(C_1-C_6)$ alkanoyloxy.

20

36. The compound of claim 24 wherein  $R_8$  is heteroaryl, optionally substituted by 1, 2, or 3 substituents independently selected from the group consisting of  $(C_1-C_6)$ alkyl,  $(C_3-C_6)$ cycloalkyl,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkyl,  $(C_1-C_6)$ alkoxy,  $(C_3-C_6)$ cycloalkyl $(C_1-C_6)$ alkoxy, hydroxy, halo, nitro, cyano, mercapto, carboxy, hydroxy $(C_1-C_6)$ alkyl, halo $(C_1-C_6)$ alkyl, trifluoromethoxy,  $(C_1-C_6)$ alkanoyl,  $(C_1-C_6)$ alkoxycarbonyl,  $(C_1-C_6)$ alkylthio, and  $(C_1-C_6)$ alkanoyloxy.

25

37. The compound 5,6-dibromo-2-[2'-(benzimidazol-5''-yl)benzimidazol-5'yl] benzimidazole, or a pharmaceutically acceptable salt thereof.

38. A pharmaceutical composition comprising a compound of any one of  
5 claims 24 to 37, in combination with a pharmaceutically acceptable carrier.

39. A therapeutic method comprising treating cancer by administering to a mammal in need of such therapy, an effective amount of a compound of claim 24.

10

40. A method comprising inhibiting cancer cells by contacting said cancer cells with an effective amount of a compound of claim 24.

41. A therapeutic method comprising treating fungal infection by  
15 administering to a mammal afflicted with a fungal infection, an effective antifungal amount of a compound of claim 1 or claim 24.

42. A compound of any one of claims 1 to 20, or 24 to 37, for use in medical therapy.

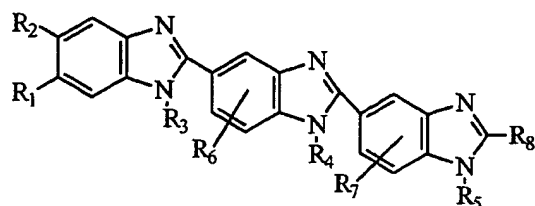
20

43. The compound of claim 42 wherein the medical therapy is the treatment of cancer.

44. The use of a compound of any one of claims 1 to 20, or 24 to 37, for the  
25 manufacture of a medicament useful for the treatment of cancer.

45. The use of a compound of any one of claims 1 to 20, or 24 to 37, for the manufacture of a medicament useful for the treatment of fungal infection.

FIGURE 1



Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>7</sub>	R <sub>9</sub>
1	H	Phenyl	H	H	H	H	H	H
2	H	Phenyl	H	H	H	H	H	OH
3	H	Phenyl	H	H	H	H	H	SH
4	H	Phenyl	H	H	H	H	H	NH <sub>2</sub>
5	H	Phenyl	H	H	H	H	H	CF <sub>3</sub>
6	H	Phenyl	H	H	H	H	H	CH <sub>3</sub>
7	H	Phenyl	H	H	H	H	H	CH <sub>2</sub> OH
8	H	Phenyl	H	H	H	H	H	CH <sub>2</sub> NHCOC <sub>6</sub> H <sub>5</sub>
9	H	Phenyl	H	H	H	H	H	CH <sub>2</sub> CH <sub>3</sub>
10	H	Phenyl	H	H	H	H	H	(CH <sub>2</sub> ) <sub>2</sub> OH
11	H	Phenyl	H	H	H	H	H	(CH <sub>2</sub> ) <sub>2</sub> NH <sub>2</sub>
12	H	Phenyl	H	H	H	H	H	(CH <sub>2</sub> ) <sub>2</sub> NHCOCH <sub>3</sub>
13	H	Phenyl	H	H	H	H	H	(CH <sub>2</sub> ) <sub>2</sub> OCH <sub>3</sub>
14	H	Phenyl	H	H	H	H	H	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>
15	Phenyl	OCH <sub>3</sub>	H	H	H	H	H	H
16	Phenyl	Phenyl	H	H	H	H	H	H
17	H	Br	H	H	H	H	H	H
18	H	Br	H	H	H	H	H	OH
19	H	Br	H	H	H	H	H	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>
20	Br	Br	H	H	H	H	H	H
21	OCH <sub>3</sub>	Br	H	H	H	H	H	H
22	Br	Br	H	H	H	H	H	CF <sub>3</sub>
23	p-Cl-phenyl	H	H	H	H	H	H	CF <sub>3</sub>

Figure 2

Compounds	Topo-I mediated DNA cleavage	Cytotoxicity ( $\mu\text{g/ml}$ ) RPMI 8402	Cytotoxicity ( $\mu\text{g/ml}$ ) CPT-K5	Cytotoxicity ( $\mu\text{g/ml}$ ) U937	Cytotoxicity ( $\mu\text{g/ml}$ ) U/CR
1	0.01	0.07	0.33	0.025	0.023
2	0.1	0.09	0.28	0.02	0.03
3	0.01	0.06	0.33	0.025	0.02
4	1.0	>10	>10		
5	<0.01	0.02	>10	0.006	0.01
6	0.02	0.11	N.D.	N.D.	N.D.
7	0.1	0.09	>10	0.025	0.035
8	0.1	5	>10	5.5	5.5
9	0.01	0.11	N.D.	N.D.	N.D.
10	0.005	1.2	>10	0.45	0.5
11	0.1	4	>10	3	3.5
12	0.01	0.05	>50	0.3	0.035
13	0.1	0.18	>10	0.055	0.06
14	0.005	0.06	N.D.	N.D.	N.D.
15	N.D.	0.66	>10	0.39	0.46
16	0.01	4.3	>10	N.D.	N.D.
17	0.01	1.4	N.D.	N.D.	N.D.
18	<0.01	1.5	0.09	0.7	0.75
19	0.01	0.3	2.0	0.3	0.25
20	0.005	0.2	0.25	0.06	0.15
21	0.01	3.66	>10	2.84	5.46
22	0.01	0.63	4.94	0.55	0.49
23	0.01	0.79	6.1	0.066	0.053



# INTERNATIONAL SEARCH REPORT

II. National Application No

PCT/US 99/02966

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D235/18 C07D235/26 C07D235/28 C07D235/30 A61K31/415  
C07D491/04 C07D235/02 //(C07D491/04,317:00,235:00)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 36612 A ( RUTGERS ,THE STATE UNIVERSITY OF NEW JERSEY) 21 November 1996 see claims	1-45
Y	SUN Q ET AL: "SYNTHESIS AND EVALUATION OF TERBENZIMIDAZOLES AS TOPOISOMERASE I INHIBITORS" JOURNAL OF MEDICINAL CHEMISTRY, vol. 38, no. 18, 1 September 1995, pages 3638-3644, XP002010635 cited in the application see the whole document	1-45

-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

17 May 1999

Date of mailing of the international search report

01/06/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Henry, J

4 0 2 4

PCT/US 99/02966

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>PILCH D S ET AL: "A TERBENZIMIDAZOLE THAT PREFERENTIALLY BINDS AND CONFORMATIONALLY ALTERS STRUCTURALLY DISTINCT DNA DUPLEX DOMAINS: A POTENTIAL MECHANISM FOR TOPOISOMERASE I POISONING"            PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,            vol. 94, no. 2, 1 December 1997, pages 13565-13570, XP002066301            see the whole document</p>	1-45
Y	<p>KIM J S ET AL: "TERBENZIMIDAZOLES: INFLUENCE OF 2"-, 4-, AND 5-SUBSTITUENTS ON CYTOTOXICITY AND RELATIVE POTENCY AS TOPOISOMERASE I POISONS"            JOURNAL OF MEDICINAL CHEMISTRY,            vol. 40, no. 18, 1 September 1997, pages 2818-2824, XP002066302            see the whole document</p>	1-45
Y	<p>GOLDMAN G H ET AL: "DIFFERENTIAL POISONING OF HUMAN AND ASPERGILLUS NIDULANS DNA TOPOISOMERASE I BY- AND TERBENZIMIDAZOLES"            BIOCHEMISTRY,            vol. 36, no. 21, 27 May 1997, pages 6488-6494, XP002066842            see the whole document</p>	1-45
Y	<p>A.Y. CHEN ET AL: "A NEW MAMMALIAN DNA TOPOISOMERASE I POISON HOECHST 33342: CYTOTOXICITY AND DRUG RESISTANCE IN HUMAN CELL CULTURES"            CANCER RESEARCH,            vol. 53, no. 6, 15 March 1993, pages 1332-1337, XP002010634            cited in the application            see the whole document</p>	1-45
X,P	<p>WO 98 31673 A ( RUTGERS , THE STATE UNIVERSITY OF NEW JERSEY) 23 July 1998            see claims</p>	1-45

# INTERNATIONAL SEARCH REPORT

international application No.

PCT/US 99/02966

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 22,23 and 39-41  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 22,23 and 39-41  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/02966

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9636612 A	21-11-1996	US 5807874 A	15-09-1998
		US 5767142 A	16-06-1998
		AU 5746696 A	29-11-1996
		CA 2221248 A	21-11-1996
		CZ 9703631 A	13-05-1998
		EP 0839140 A	06-05-1998
		NZ 307959 A	26-08-1998
		PL 324034 A	11-05-1998
WO 9831673 A	23-07-1998	US 5770617 A	23-06-1998
		AU 6132798 A	07-08-1998